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UNITED STATES DEPARTMENT OF COMMERCE

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February 27, 2006

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FILING DATE: *March 31, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US04/32599*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US60/558,504*

UNITED STATES PATENT AND TRADEMARK OFFICE · DEPARTMENT OF COMMERCE

Certified by

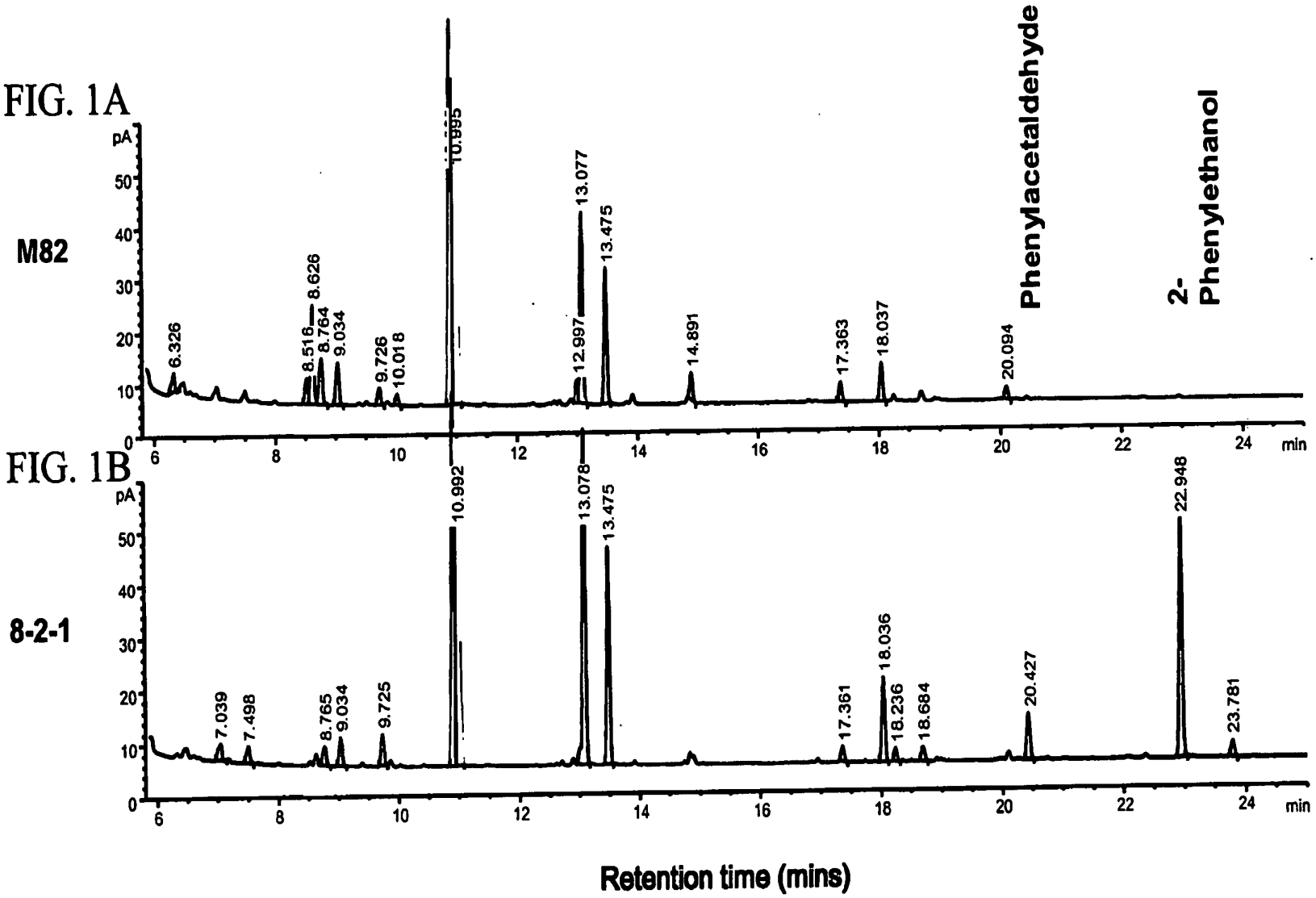
Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

Abstract of the Disclosure

The subject invention concerns polynucleotides encoding a plant 2-phenylethanol dehydrogenase enzyme. In one embodiment, the polynucleotide encodes a tomato 2-phenylethanol dehydrogenase. The subject invention also concerns polynucleotides encoding a plant phenylalanine decarboxylase enzyme. In one embodiment, the polynucleotide encodes a tomato phenylalanine decarboxylase. The subject invention also concerns 2-phenylethanol dehydrogenase polypeptides and phenylalanine decarboxylase polypeptides encoded by polynucleotides of the present invention. The subject invention also concerns methods for providing a plant with an increased flavor and aroma volatile. Plants can be transformed with one or more polynucleotide of the present invention. The subject invention also concerns these transformed plant cells, plant tissue, and plants and transgenic progeny thereof.

FIG. 1A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9.726
10.018
10.995
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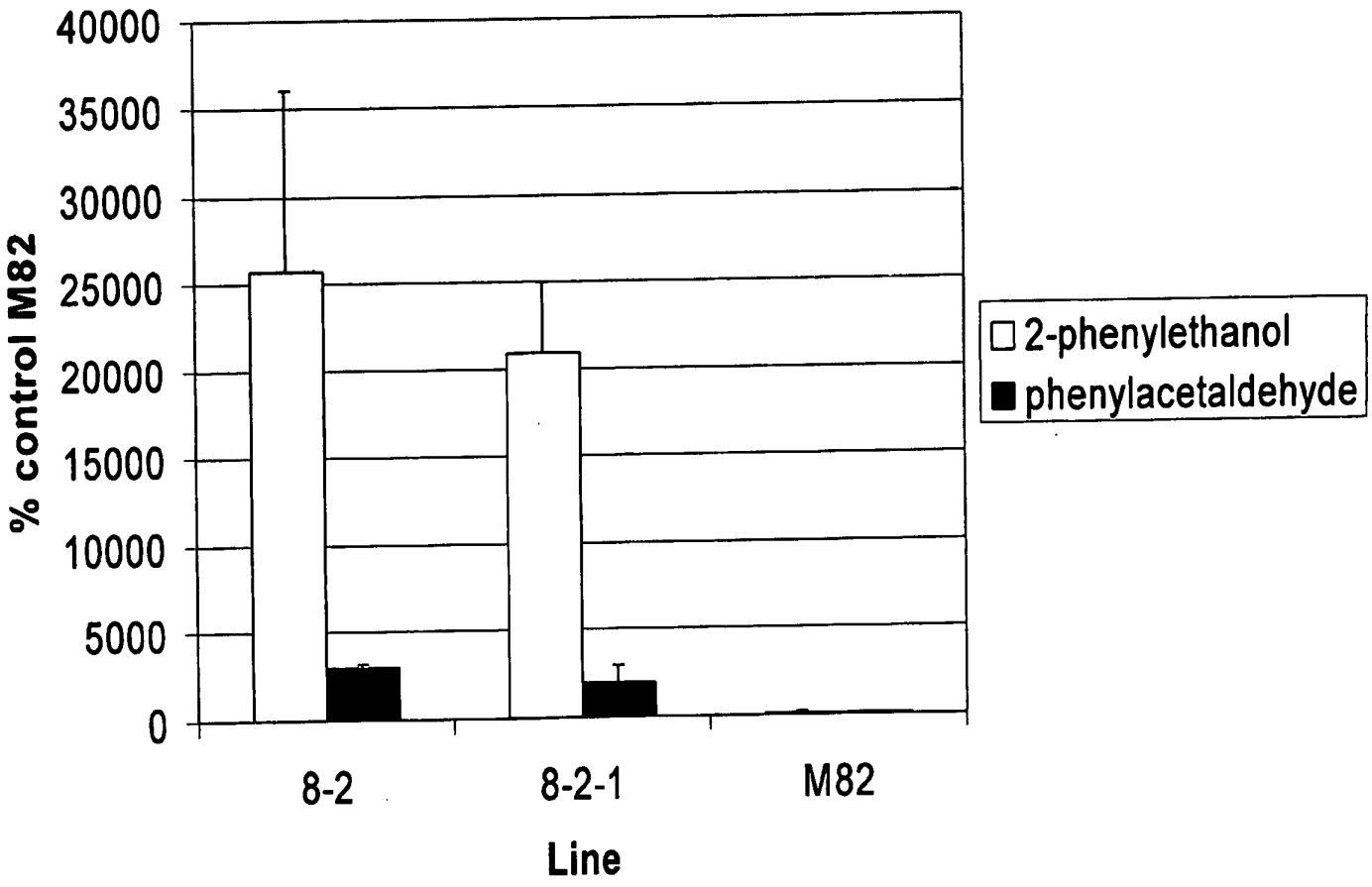
13.475
14.891
17.363
18.037
20.094
Phenylacetaldehyde
2-Phenylethanol
min
8-2-1
7.039
7.498
8.765
9.034
9.725
10.992
13.078
13.475
17.361
18.036
18.236
18.684
20.427
22.948
23.781
Retention time (mins)

FIG. 1B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30000
25000
20000
15000
10000
5000
0
% control M82
8-2
8-2-1
M82
Line
2-phenylethanol
phenylacetaldehyde

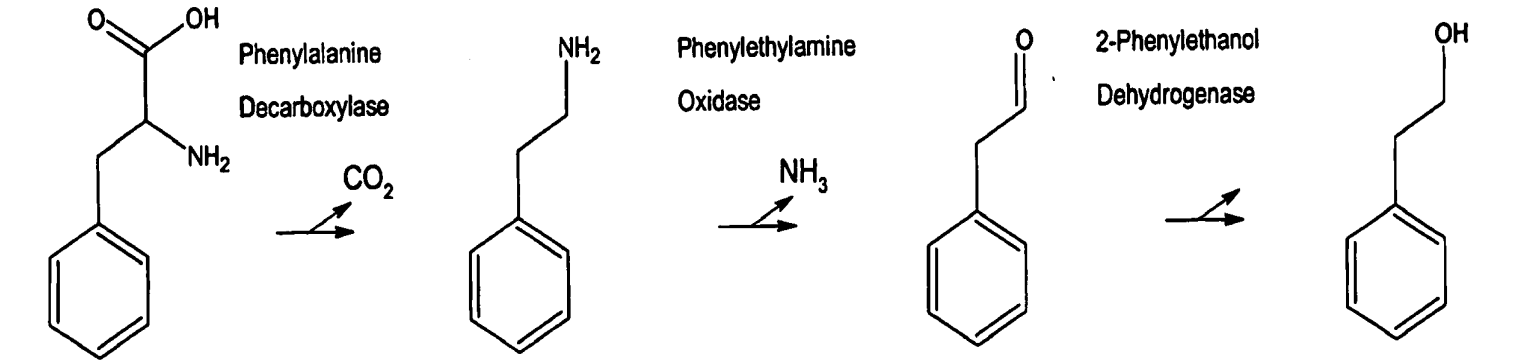
FIG. 2

```
   1  GCCCTTCTAA TACGACTCAC TATAGGGCAA GCAGTGGTAA CAACGCAGAG
  51  TACGCGGGGG AAGGATAATC TCTCAAATTA CTTTCTTTTT TTTTCCTATC
 101  AATTCTTTAT ACCAAAATAA TATTATTGTT TTTTTCTCCT CTGTTTCTGC
 151  TTCGTATTTT TGCTGAGAGA AATGAGTGTG ACAGCGAAAA CAGTGTGTGT
 201  AACAGGAGCT TCAGGTTACA TAGCTTCATG GCTAGTCAAA TTCTTGCTAC
 251  ATAGTGGTTA CAATGTGAAG GCTTCTGTTC GTGATCCAAA TGATCCCAAG
 301  AAAACGCAGC ACTTGCTTTC TCTTGGTGGG GCCAAGGAGA GGCTTCACTT
 351  GTTCAAAGCA AACCTATTAG AAGAAGGTTC ATTTGATGCT GTAGTTGATG
 401  GATGTGAAGG TGTATTCCAT ACAGCGTCTC CTTTTTACTA CTCTGTTACA
 451  GACCCACAGG CTGAATTACT TGATCCTGCT GTTAAGGGAA CACTCAATCT
 501  TCTCGGGTCA TGTGCCAAAG CACCATCAGT AAAACGAGTT GTTTTAACGT
 551  CTTCCATAGC TGCAGTTGCT TACAGTGGTC AGCCTCGGAC ACCTGAGGTT
 601  GTGGTTGATG AGAGCTGGTG GACCAGTCCA GACTACTGCA AAGAAAAACA
 651  GCTCTGGTAT GTCCTCTCAA AGACATTGGC TGAGGATGCT GCGTGGAAGT
 701  TTGTGAAGGA GAAAGGCATT GATATGGTTG TAGTAAACCC TGCTATGGTT
 751  ATTGGTCCTC TGTTACAGCC TACACTTAAT ACCAGTTCTG CTGCAGTCTT
 801  GAGCTTGGTA AATGGTGCTG AGACATACCC AAATTCCTCT TTTGGGTGGG
 851  TTAACGTGAA AGATGTTGCA AATGCACATA TTCTTGCATT TGAGAACCCT
 901  TCAGCTAATG GGAGATACTT AATGGTTGAG AGGGTTGCAC ACTATTCTGA
 951  TATATTGAAG ATATTGCGTG ACCTTTATCC TACTATGCAA CTTCCAGAAA
1001 AGTGTGCTGA TGACAACCCA TTGATGCAAA ATTATCAAGT ATCAAAGGAG
1051 AAGGCAAAAA GCTTGGGTAT TGAGTTTACT ACCCTTGAAG AAAGCATCAA
1101 AGAAACTGTT GAAAGTTTGA AGGAAAAGAA GTTTTTTGGA GGTTCATCTT
1151 CTATGTAAAA GGCTTCTCAA AGCTTTTATG GTTTTGTTGA ACAATACTAC
1201 CCACCCCACC CTACCCTACA CACTTTTTTT TTTTACTTCT TTTAGCTAAT
1251 TATAGAATCA AGAAGTCGAA TGGTATATCC GTTAATAAAT TTCGATCAGA
1301 TGAGGTTGAA ATTTGTTCTA TATCTAGAGA TTTTTACAGA CTGGTTTGAT
1351  AGAAAAAAAA AAAAAA
```

FIG. 3A

```
  1  MSVTAKTVCV TGASGYIASW LVKFLLHSGY NVKASVRDPN DPKKTQHLLS
 51  LGGAKERLHL FKANLLEEGS FDAVVDGCEG VFHTASPFYY SVTDPQAELL
101  DPAVKGTLNL LGSCAKAPSV KRVVLTSSIA AVAYSGQPRT PEVVVDESWW
151  TSPDYCKEKQ LWYVLSKTLA EDAAWKFVKE KGIDMVVVNP AMVIGPLLQP
201  TLNTSSAAVL SLVNGAETYP NSSFGWVNVK DVANAHILAF ENPSANGRYL
251  MVERVAHYSD ILKILRDLYP TMQLPEKCAD DNPLMQNYQV SKEKAKSLGI
301  EFTTLEESIK ETVESLKEKK FFGGSSSM
```

FIG. 3B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NH2
Phenylalanine
Decarboxylase
CO2
NH2
Phenylethylamine
Oxidase
NH3
O
2-Phenylethanol
Dehydrogenase
OH
Phenylalanine
Phenylethylamine
Phenylacetaldehyde
2-Phenylethanol

FIG. 4

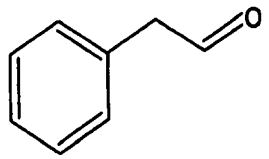


FIG. 5A-1

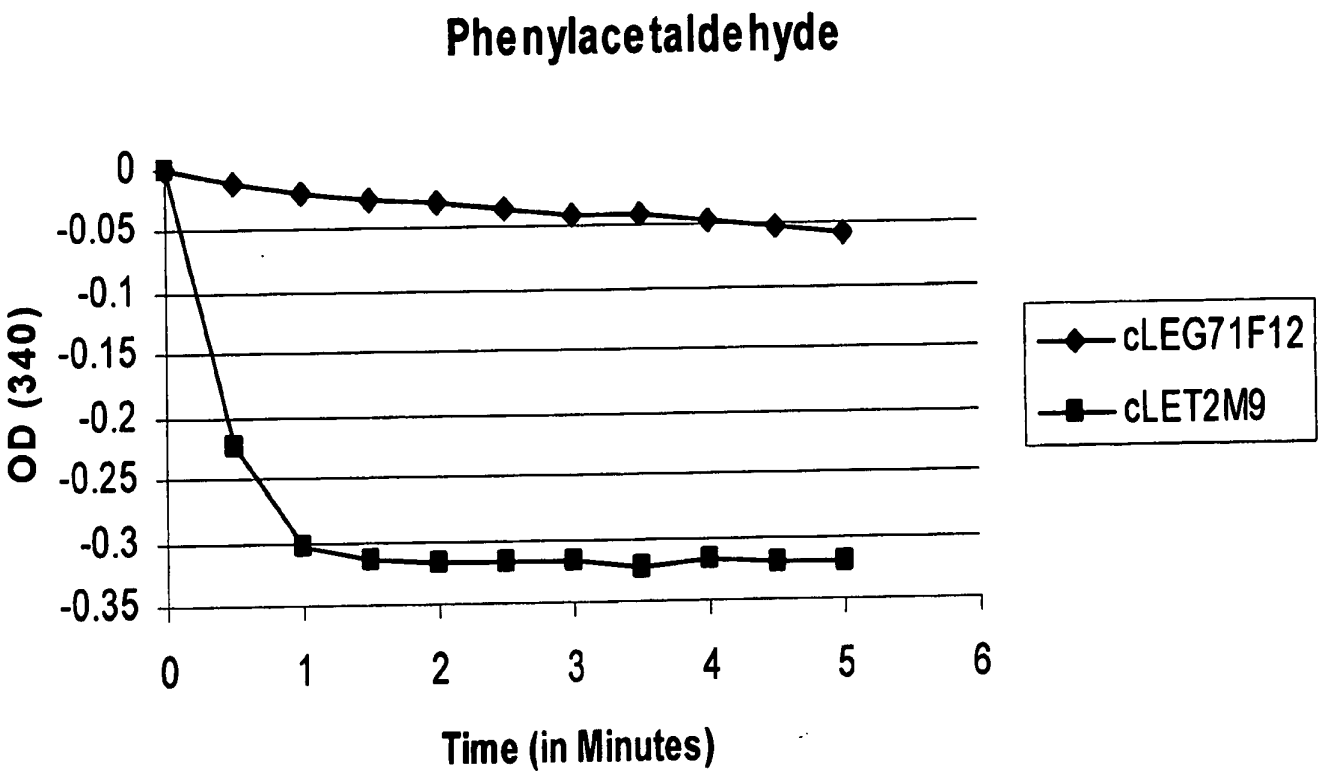


FIG. 5A

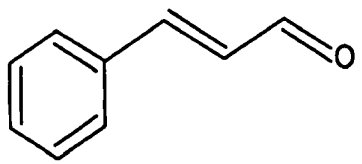


FIG. 5B-1

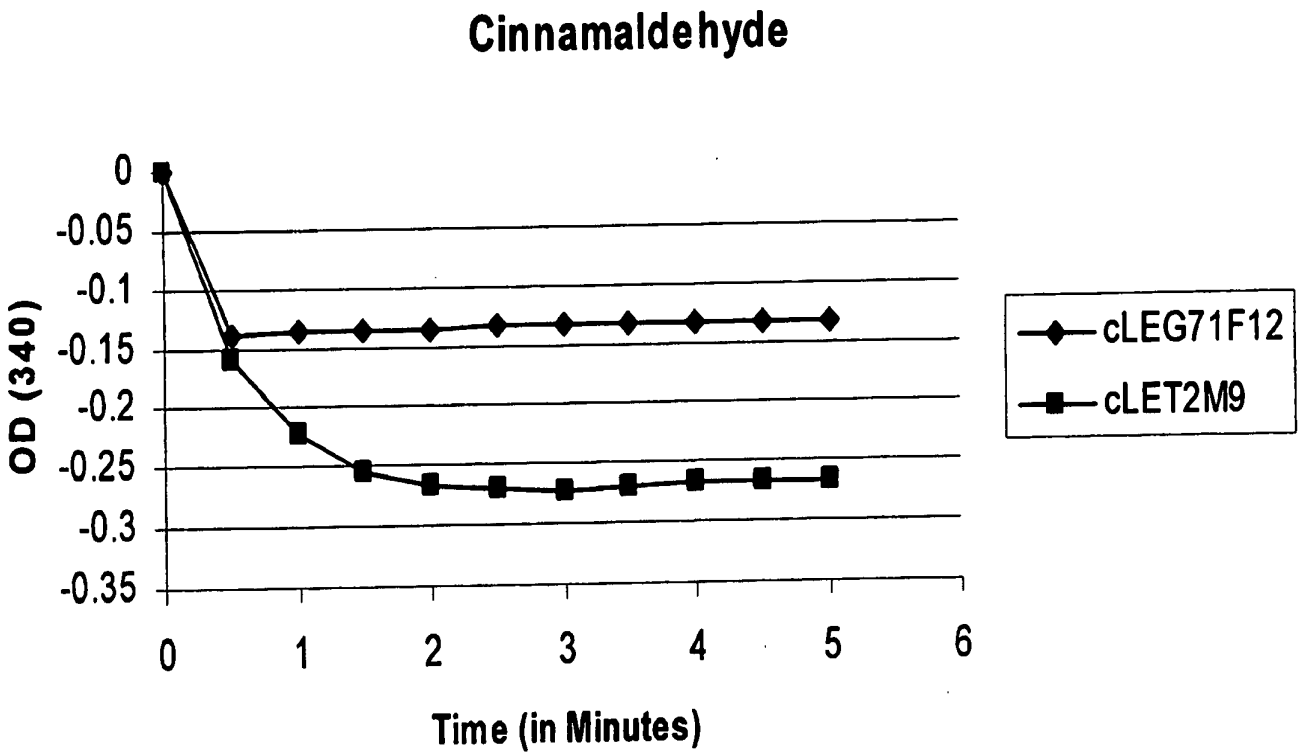


FIG. 5B

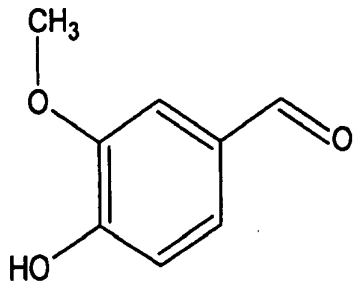


FIG. 5C-1

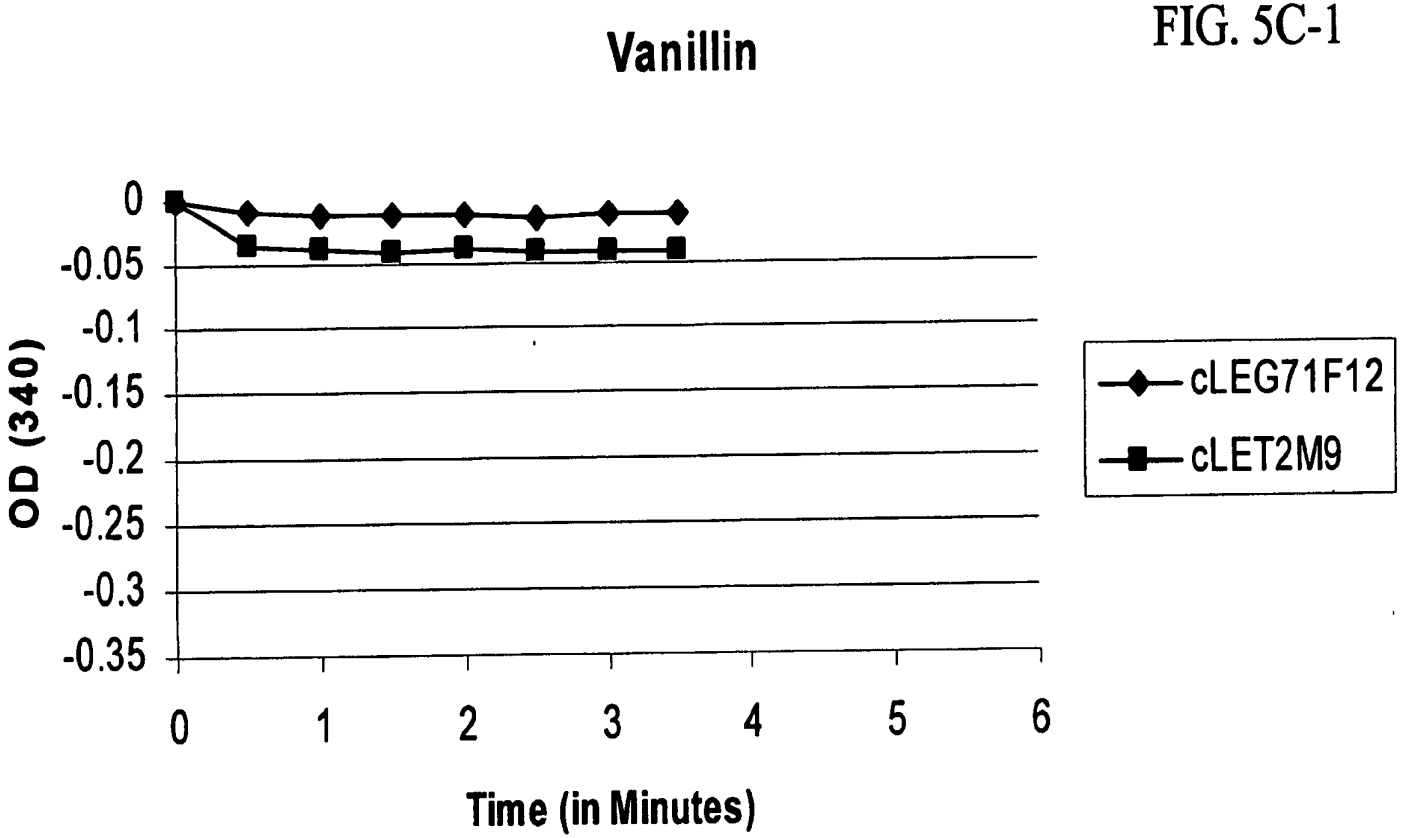
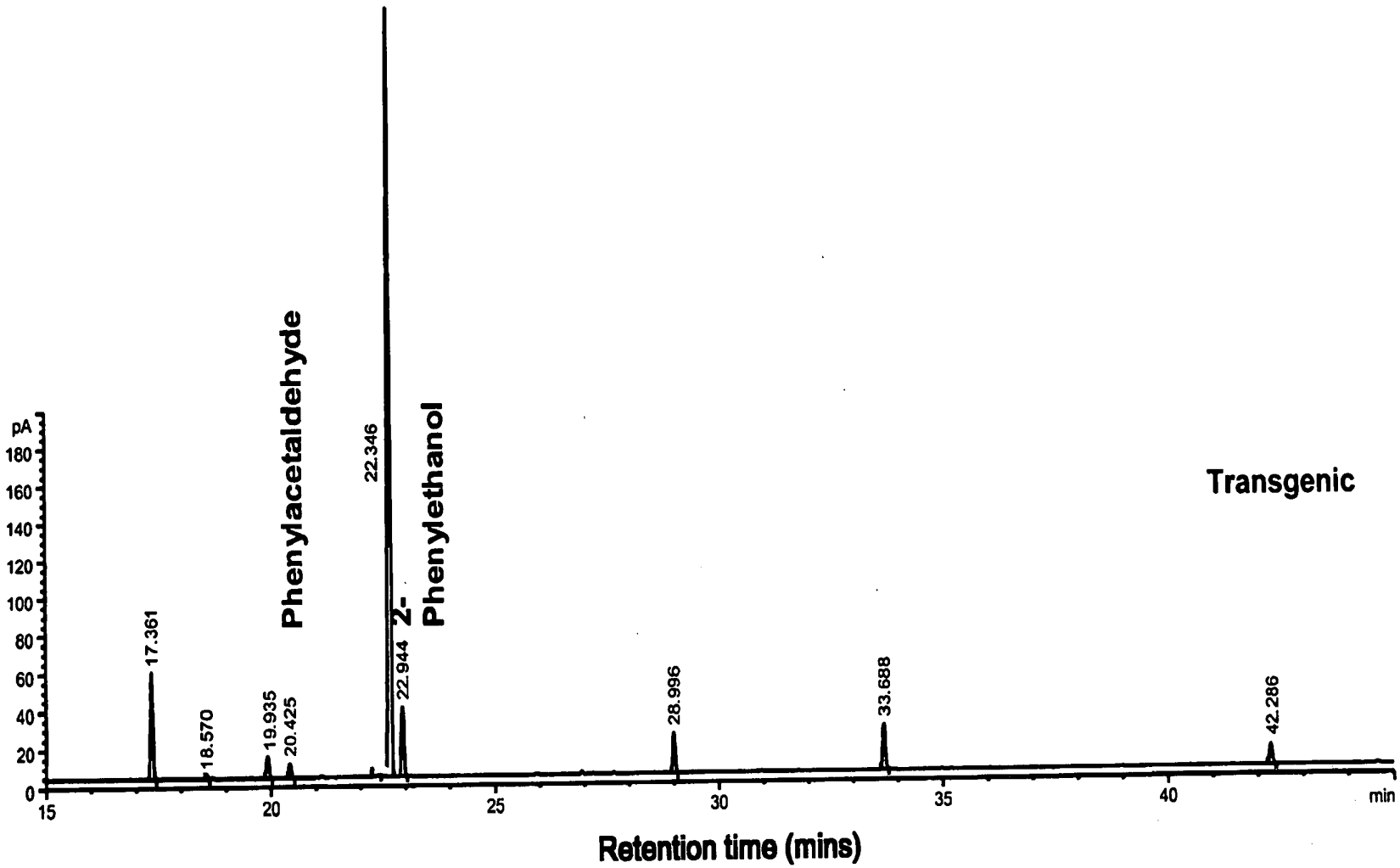


FIG. 5C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140
120
100
80
60
40
20
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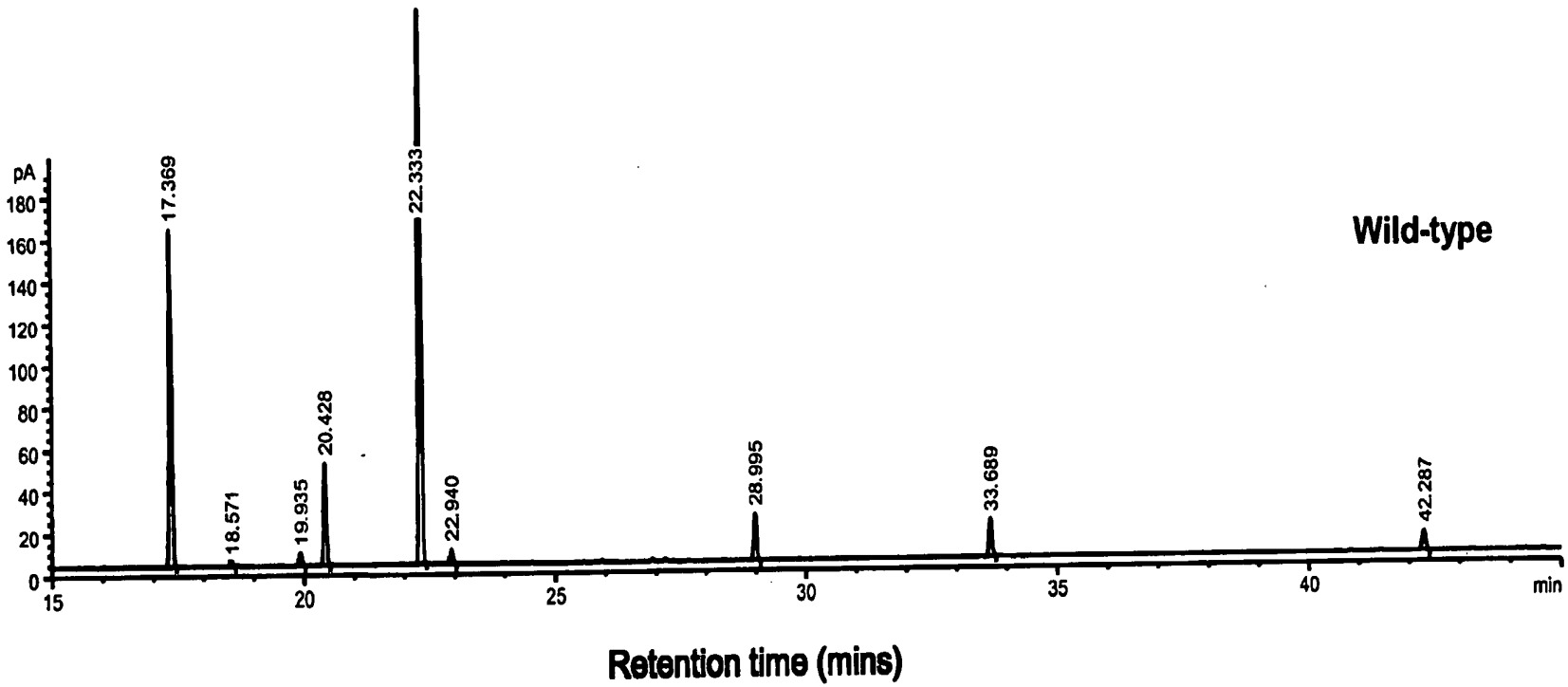
18.570
19.935
20.425
Phenylacetaldehyde
22.346
22.944
2-
Phenylethanol
28.996
33.688
42.286
Transgenic
15
20
25
30
35
40
min
Retention time (mins)

FIG. 6A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80
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19.935
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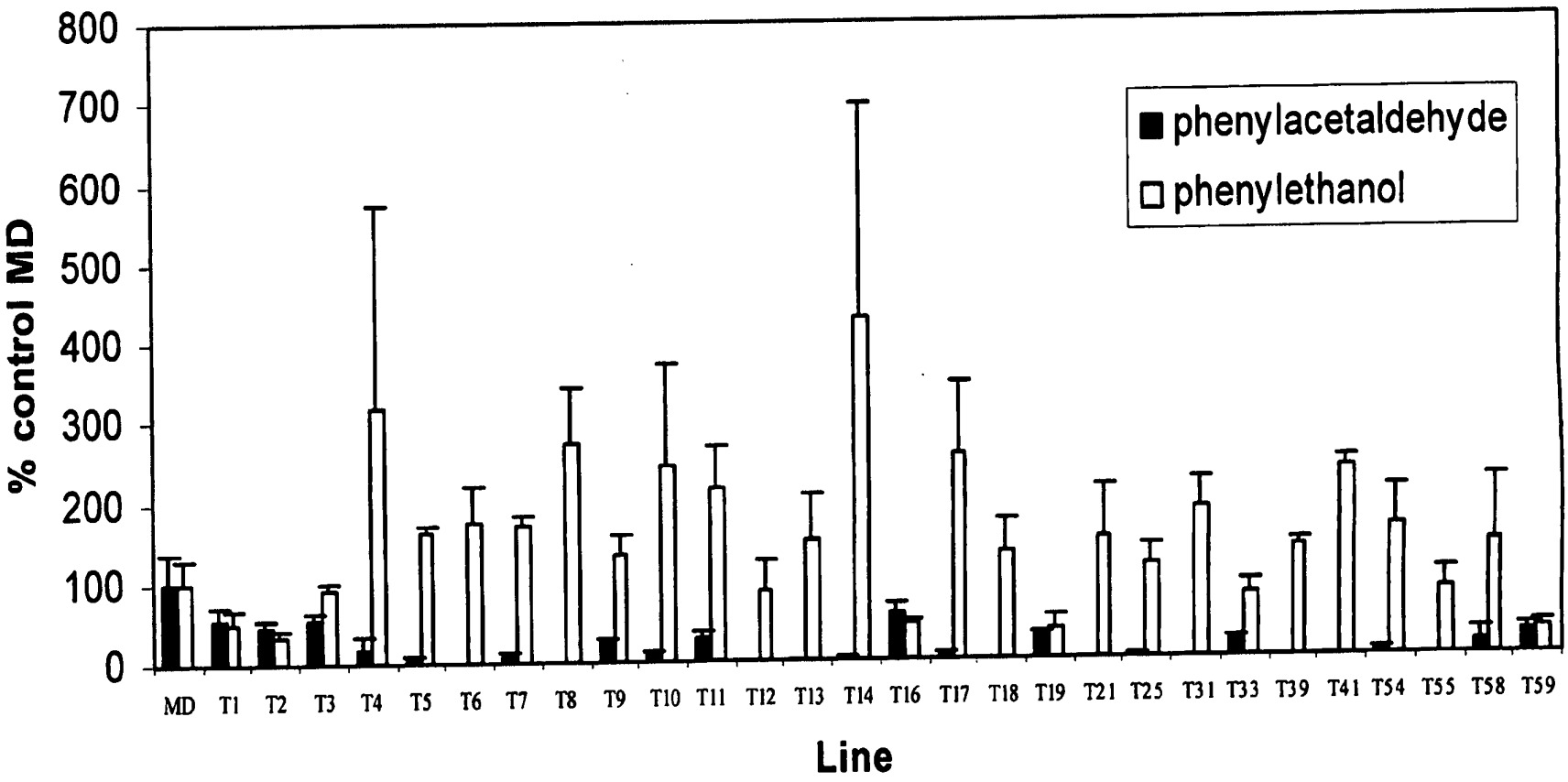
22.333
22.940
28.995
33.689
42.287
15
20
25
30
35
40
min
Wild-type
Retention time (mins)

FIG. 6B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MD T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12 T13 T14 T16 T17 T18 T19 T21 T25 T31 T33 T39 T41 T54 T55 T58 T59
Line

FIG. 7

```
1    ATGGGAAGTT TATCATTTGA GAAGGATTTT GAGCCATCAG CAATTACTCC
51   AAGAGGATTA GCACCACCTG GATTAATTGT AAATGGTGAT TTTGGTGAAA
101  TGATGAGACT TAAGGTGTCA TCAACACCAA CAACACCAAG AAAAAACTTG
151  AATCTTTCAG TGACGGAGCC AGGAAAAAAT GATGGACCTA GTTTGGATTG
201  TACATTGATG AATTATATTG ATACACTCAC CCAACGTATC AACTATCATA
251  TCGGTTATCC AGTTAACATA TGTTATGAGC ACTATGCTAA TTTAGCCCCA
301  CTTTTACAAT TTCATTTAAA TAATTGTGGT GATCCATTTC TTCAAAATAC
351  TGTGGATTTT CATTCAAAGG ATTTTGAAGT GGCTGTTTTA AATTGGTTTG
401  CTGATTTATG GGAAATTGAA AGAGATCAAT ATTGGGGCTA TGTAACAAAT
451  GGTGGTACTG AAGGAAATTT ACATGGCATT TTGGTTGGGA GAGAATTGTT
501  TCCAGATGGA ATTTTATATG CATCAAAAGA CTCTCATTAC TCAGTGGCTA
551  AGGCAGCAAT GATGTATAGA ATGGATTTTG AAAATATTAA CGCATCAATA
601  AATGGAGAAA TCGATTATTC TGATTTGAAA GTTAAATTAC TTCAAAACAA
651  GGGAAAACCA GCGATAATTA ATGTTACAAT TGGCACTACT TTTAAAGGAG
701  CTGTTGATGA TCTTGATGTT ATTCTTCAAA TACTTGAAGA GTGTGGTTAC
751  ACACGAGATC AATTTTATAT TCATTGTGAT GCAGCACTAA ATGGACTTAT
801  TATTCCTTTT ATTAAAAATA TGATTACTTT CAAGAAGCCA ATTGGAAGTG
851  TGACAATTTC TGGTCACAAG TTTTTGGGAT GTCCAATGCC TTGTGGAGTT
901  CAAATAACAA GGAAAAGTTA CATTAATAAC CTTTCGAGAA GAGTCGAATA
951  TATTGCTTCT GTGGATGCTA CAATTTCTGG AAGTCGAAAT GGTTTGACTC
1001 CGATCTTCTT ATGGTACAGT ATAAGTGCTA AAGGTCAAAT TGGTTTTCAG
1051 AAAGACGTTA AGAGATGTTT TGACAATGCT AAGTACTTGA AAGACCGTCT
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1151 TCCTCGAGAG GCCTCGTGAC CATGAATTCG TTCGTCGTTg GCAATTATCT
1201 TGTGTGAGAG ATATGGCACA TGTTATTGTT ATGCCAGGCA TAACTAGAGA
1251 AACTCTTGAT GGTTTTATTA ATGATTTGCT TCAACAAAGG AAAAAATGGT
1301 ATCAAGATGG AAGAATTAGC CCTCCTTGTG TTGCAAATGA TATTGGTGCT
1351 CAAAATTGTG CTTGCTCTTA TCATAAAATT GATTACATTA TTGCTTAG
```

FIG. 8A

```
  1  MGSLSFEKDF EPSAITPRGL APPGLIVNGD FGEMMRLKVS STPTTPRKNL
 51  NLSVTEPGKN DGPSLDCTLM NYIDTLTQRI NYHIGYPVNI CYEHYANLAP
101  LLQFHLNNCG DPFLQNTVDF HSKDFEVAVL NWFADLWEIE RDQYWGYVTN
151  GGTEGNLHGI LVGRELFPDG ILYASKDSHY SVAKAAMMYR MDFENINASI
201  NGEIDYSDLK VKLLQNKGKP AIINVTIGTT FKGAVDDLDV ILQILEECGY
251  TRDQFYIHCD AALNGLIIPF IKNMITFKKP IGSVTISGHK FLGCPMPCGV
301  QITRKSYINN LSRRVEYIAS VDATISGSRN GLTPIFLWYS ISAKGQIGFQ
351  KDVKRCFDNA KYLKDRLQQA GISVMLNELS IIVVLERPRD HEFVRRWQLS
401  CVRDMAHVIV MPGITRETLD GFINDLLQQR KKWYQDGRIS PPCVANDIGA
451  QNCACSYHKI DYIIA
```

FIG. 8B

```
   1  ATGGGTAGTC TCTCACTTGA AATGGATTTT GAGCCATCAC CCATGACACC
  51  CAGAAGTTTA GCAGCGATGA CACCTAGAAG TTTAGCGCGA CGACGATTGT
 101  TTCCGAACGT GGACAACAAG AAACAGAAAA TGGCACAACC AGGTGCAGGA
 151  CCAAGGAAGA ACTTGGAACT TGAGGTCATG GAGCCTGCAT TGAAGAATGA
 201  TGGTCCTTCT TTGGACACTA TCTTGGTTAA TTATTTGGAC ACACTTACAC
 251  AACGAGTCAA TTATCATTTA GGTTATCCAG TCAACATATG TTATGATCAC
 301  TATGCAACGC TAGCACCACT TTTGCAGTTT CACCTAAACA ATTGTGGTGA
 351  TCCTTTCCTA CAAAATACTG TCGATTTCCA TTCTAAAGAC TTTGAAGTGG
 401  CTGTTTTGAA TTGGTTTGCA AAACTTTGGG AAATTGAAAA GGATCAATAT
 451  TGGGGATATG TTACCAATGG TGGCACCGAA GGCAATCTCC ATGGTATTTT
 501  GTTAGGGAGA GAGCTACTTC CTGAAGGAAT ATTATATGCA TCAAAAGACT
 551  CTCATTACTC AGTATTCAAA GCTGCAAGAA TGTATAGAAT GGATTCAGAA
 601  ACAATCAACA CATCAGTAAA TGGAGAGATG GATTATTCAG ATTTAAGAGC
 651  AAAGTTACTT CAAAATAAGG ATAAACCAGC TATTATAAAT GTCACAATTG
 701  GAACTACATT CAAAGGAGCA ATCGATGACC TGGATGTTAT TCTTGAAATA
 751  CTCAAAGAAT GTGGCTATTC ACAAGATCGA TTTTACATTC ACTGTGATGC
 801  AGCACTATGT GGTCTTATGA CCCCTTTTAT AAACAATATG ATTAGTTTCA
 851  AGAAGCCAAT TGGAAGTGTC ACAATTTCTG GACACAAGTT TTTGGGATGT
 901  CCAATGCCTT GTGGTGTCCA AATAACAAGA AAAAGCTACA TCAATAATCT
 951  CTCAACAAAT GTGGAATACA TTGCTTCTGT GGATGCCACT ATTTCTGGTA
1001  GCCGTAACGG TTTAACTCCA ATTTTCTTAT GGTATAGCTT GAGCGCAAAA
1051  GGTCAAGTTG GACTTCAAAA GGATGTTAAA AGATGTCTCG ACAATGCCAA
1101  ATATTTGAAA GATCGTCTTC AACAAGCAGG GATAAGTGTC ATGCTGAATG
1151  AGCTAAGCAT CATAGTTGTA CTTGAAAGGC CTCGTGACCA TGAATTTGTG
1201  CGTCGTTGGC AACTCTCATG CGTCAAGGAT ATGGCACATG TTATTGTGAT
1251  GCCAGGAATC ACACGAGAAA TGCTTGACAA CTTCATGAGT GAATTAGTGC
1301  AACAAAGAAA AGTATGGTAT CAAAATGGAA AGACTGATCC TCCTTGTGTT
1351  GGAGAGGATA TTGGTGCTCA AAATTGTGCA TGCTCTTATC ATAAGATTGA
1401  CTACATCTGT CCTTAG
```

FIG. 9A

```
  1   MGSLSLEMDF EPSPMTPRSL AAMTPRSLAR RRLFPNVDNK KQKMAQPGAG
 51   PRKNLELEVM EPALKNDGPS LDTILVNYLD TLTQRVNYHL GYPVNICYDH
101   YATLAPLLQF HLNNCGDPFL QNTVDFHSKD FEVAVLNWFA KLWEIEKDQY
151   WGYVTNGGTE GNLHGILLGR ELLPEGILYA SKDSHYSVFK AARMYRMDSE
201   TINTSVNGEM DYSDLRAKLL QNKDKPAIIN VTIGTTFKGA IDDLDVILEI
251   LKECGYSQDR FYIHCDAALC GLMTPFINNM ISFKKPIGSV TISGHKFLGC
301   PMPCGVQITR KSYINNLSTN VEYIASVDAT ISGSRNGLTP IFLWYSLSAK
351   GQVGLQKDVK RCLDNAKYLK DRLQQAGISV MLNELSIIVV LERPRDHEFV
401   RRWQLSCVKD MAHVIVMPGI TREMLDNFMS ELVQQRKVWY QNGKTDPPCV
451   GEDIGAQNCA CSYHKIDYIC P
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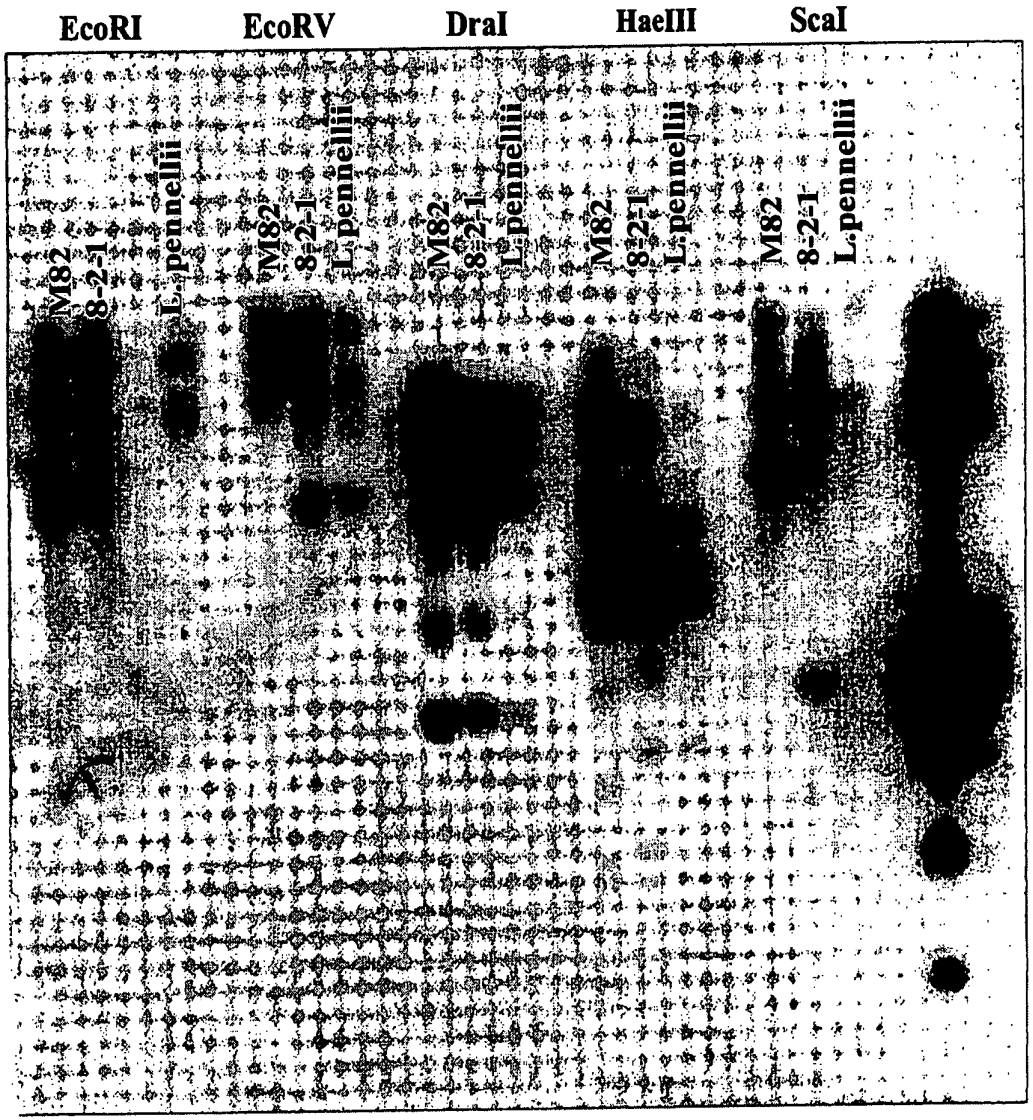
FIG. 9B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M82
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M82
8-2-1
L.pennellii

FIG. 10

```
1    ATGGGTAGTC TCTCACTTGA AATGGATTTT GAGCCATCAC CTATGACACC
51   CAGAAGTTTA GCAGCGATGA CACCTAGAAG TTTAGCGCGG CGAAGATTGT
101  TTCCCAATGT GGACAACAAA AAACAAAAGG TGCAACAATC AGGTGCAGGG
151  CCAAGGAAGA ACTTACAACT TGAAGTCATG GAACCTGCAT TGAACAATGC
201  TGGTCCCTCT TTGGACACTA TATTGGTCAA TTATTTAGAC ACACTTACAC
251  AACGAGTCAA TTATCATTTA GGTTATCCAG TCAACATTTG TTATGATCAC
301  TATGCAACTT TAGCACCACT TTTACAGTTT CACCTAAACA ATTGTGGTGA
351  TCCTTTCCTA CAAAACACTG TCGATTTCCA TTCTAAAGAC TTTGAAGTGG
401  CTGTTTTGAA TTGGTTTGCA AAACTATGGG AAATTGAAAA GGATCAATAC
451  TGGGGATATG TTACCAATGG TGGCACCGAA GGCAATCTCC ATGGTATTTT
501  GTTAGGGAGA GAGCTACTTC CTGATGGAAT ATTATATGCG TCAAAAGACT
551  CTCACTATTC GGTCTTCAAA GCTGCAAGAA TGTATAGAAT GGATTCAGAA
601  ACAATCAACA CATCAGTAAA CGGAGAGATG GATTATTCAG ATTTAAGAGC
651  AAAGTTACTT CAAAATAAGG ATAAACCAGC TATTATAAAT GTCACAATTG
701  GAACTACGTT CAAAGGAGCA ATCGATGACC TGGATGTTAT TCTTGAAACA
751  CTCAAAGAAT GTGGCTATTC GCAAGATAGG TTTTACATCC ACTGTGATGC
801  TGCACTATGT GGTCTTATGA CCCCTTTTAT AAACAATATG ATTAGTTTCA
851  AGAAGCCAAT TGGAAGTGTC ACAATTTCTG GACACAAGTT TTTGGGATGT
901  CCAATGCCTT GTGGTGTCCA AATTACAAGA AAGAGTTACA TCAATAATCT
951  CTCAACAAAT GTGGAATACA TTGCTTCTGT CGATGCCACT ATTTCTGGCA
1001 GCCGTAACGG TTTAACTCCA ATTTTCTTGT GGTATAGCTT GAGCGCAAAA
1051 GGTCAAGTTG GACTTCAAAA GGATGTTAAA AGATGTCTCG ACAATGCCAA
1101 ATATTTGAAA GATCGTCTTC AAAAAGCAGG AATAAGTGTC ATGTTAAATG
1151 AGCTTAGCAT CATAGTTGTA CTTGAAAGGC CTCGTGACCA TGAATTTGTC
1201 CGTCGTTGGC AACTCTCATG CGTCAAGGAT ATGGCACATG TTATTGTAAT
1251 GCCAGGCATC ACACGAGAAA TGCTTGACAA TTTCACGAGT GAATTAGTGC
1301 AACAAAGAAA AGTATGGTAT CAAAATGGAC AGACCAATCC TCCTTGTGTT
1351 GGAGAGGATA TTGGTGCTCA AAATTGTGCA TGCTCTTATC ATAAGATTGA
1401 CTACATCTGT CCTTAG
```

FIG. 11A

```
 1   MGSLSLEMDF EPSPMTPRSL AAMTPRSLAR RRLFPNVDNK KQKVQQSGAG
51   PRKNLQLEVM EPALNNAGPS LDTILVNYLD TLTQRVNYHL GYPVNICYDH
101  YATLAPLLQF HLNNCGDPFL QNTVDFHSKD FEVAVLNWFA KLWEIEKDQY
151  WGYVTNGGTE GNLHGILLGR ELLPDGILYA SKDSHYSVFK AARMYRMDSE
201  TINTSVNGEM DYSDLRAKLL QNKDKPAIIN VTIGTTFKGA IDDLDVILET
251  LKECGYSQDR FYIHCDAALC GLMTPFINNM ISFKKPIGSV TISGHKFLGC
301  PMPCGVQITR KSYINNLSTN VEYIASVDAT ISGSRNGLTP IFLWYSLSAK
351  GQVGLQKDVK RCLDNAKYLK DRLQKAGISV MLNELSIIVV LERPRDHEFV
401  RRWQLSCVKD MAHVIVMPGI TREMLDNFTS ELVQQRKVWY QNGQTNPPCV
451  GEDIGAQNCA CSYHKIDYIC P
```

FIG. 11B

```
                1                                                   50
Lp-cLEC73K23 MGSLSLEMDF EPSPMTPRSL AAMTPRSLAR RRLFPNVDNK KQKVQQSGAG
Le-cLEC73K23 MGSLSLEMDF EPSPMTPRSL AAMTPRSLAR RRLFPNVDNK KQKMAQPGAG
Le-cLEC75E21 MGSLSFEKDF EPSAITPRGL A...PPGLIV NGDFGEM..M RLKVSSTPTT

               51                                                  100
Lp-cLEC73K23 PRKNLQLEVM EPALNNAGPS LDTILVNYLD TLTQRVNYHL GYPVNICYDH
Le-cLEC73K23 PRKNLELEVM EPALKNDGPS LDTILVNYLD TLTQRVNYHL GYPVNICYDH
Le-cLEC75E21 PRKNLNLSVT EPG.KNDGPS LDCTLMNYID TLTQRINYHI GYPVNICYEH

              101                                                  150
Lp-cLEC73K23 YATLAPLLQF HLNNCGDPFL QNTVDFHSKD FEVAVLNWFA KLWEIEKDQY
Le-cLEC73K23 YATLAPLLQF HLNNCGDPFL QNTVDFHSKD FEVAVLNWFA KLWEIEKDQY
Le-cLEC75E21 YANLAPLLQF HLNNCGDPFL QNTVDFHSKD FEVAVLNWFA DLWEIERDQY

              151                                                  200
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Le-cLEC73K23 WGYVTNGGTE GNLHGILLGR ELLPEGILYA SKDSHYSVFK AARMYRMDSE
Le-cLEC75E21 WGYVTNGGTE GNLHGILVGR ELFPDGILYA SKDSHYSVAK AAMMYRMDFE

              201                                                  250
Lp-cLEC73K23 TINTSVNGEM DYSDLRAKLL QNKDKPAIIN VTIGTTFKGA IDDLDVILET
Le-cLEC73K23 TINTSVNGEM DYSDLRAKLL QNKDKPAIIN VTIGTTFKGA IDDLDVILEI
Le-cLEC75E21 NINASINGEI DYSDLKVKLL QNKGKPAIIN VTIGTTFKGA VDDLDVILQI
```

FIG. 12A

```
          251                                                       300
Lp-cLEC73K23 LKECGYSQDR FYIHCDAALC GLMTPFINNM ISFKKPIGSV TISGHKFLGC
Le-cLEC73K23 LKECGYSQDR FYIHCDAALC GLMTPFINNM ISFKKPIGSV TISGHKFLGC
Le-cLEC75E21 LEECGYTRDQ FYIHCDAALN GLIIPFIKNM ITFKKPIGSV TISGHKFLGC

          301                                                       350
Lp-cLEC73K23 PMPCGVQITR KSYINNLSTN VEYIASVDAT ISGSRNGLTP IFLWYSLSAK
Le-cLEC73K23 PMPCGVQITR KSYINNLSTN VEYIASVDAT ISGSRNGLTP IFLWYSLSAK
Le-cLEC75E21 PMPCGVQITR KSYINNLSRR VEYIASVDAT ISGSRNGLTP IFLWYSISAK

          351                                                       400
Lp-cLEC73K23 GQVGLQKDVK RCLDNAKYLK DRLQKAGISV MLNELSIIVV LERPRDHEFV
Le-cLEC73K23 GQVGLQKDVK RCLDNAKYLK DRLQQAGISV MLNELSIIVV LERPRDHEFV
Le-cLEC75E21 GQIGFQKDVK RCFDNAKYLK DRLQQAGISV MLNELSIIVV LERPRDHEFV

          401                                                       450
Lp-cLEC73K23 RRWQLSCVKD MAHVIVMPGI TREMLDNFTS ELVQQRKVWY QNGQTNPPCV
Le-cLEC73K23 RRWQLSCVKD MAHVIVMPGI TREMLDNFMS ELVQQRKVWY QNGKTDPPCV
Le-cLEC75E21 RRWQLSCVRD MAHVIVMPGI TRETLDGFIN DLLQQRKKWY QDGRISPPCV

          451                    472
Lp-cLEC73K23 GEDIGAQNCA CSYHKIDYIC P
Le-cLEC73K23 GEDIGAQNCA CSYHKIDYIC P
Le-cLEC75E21 ANDIGAQNCA CSYHKIDYII A
```

FIG. 12B

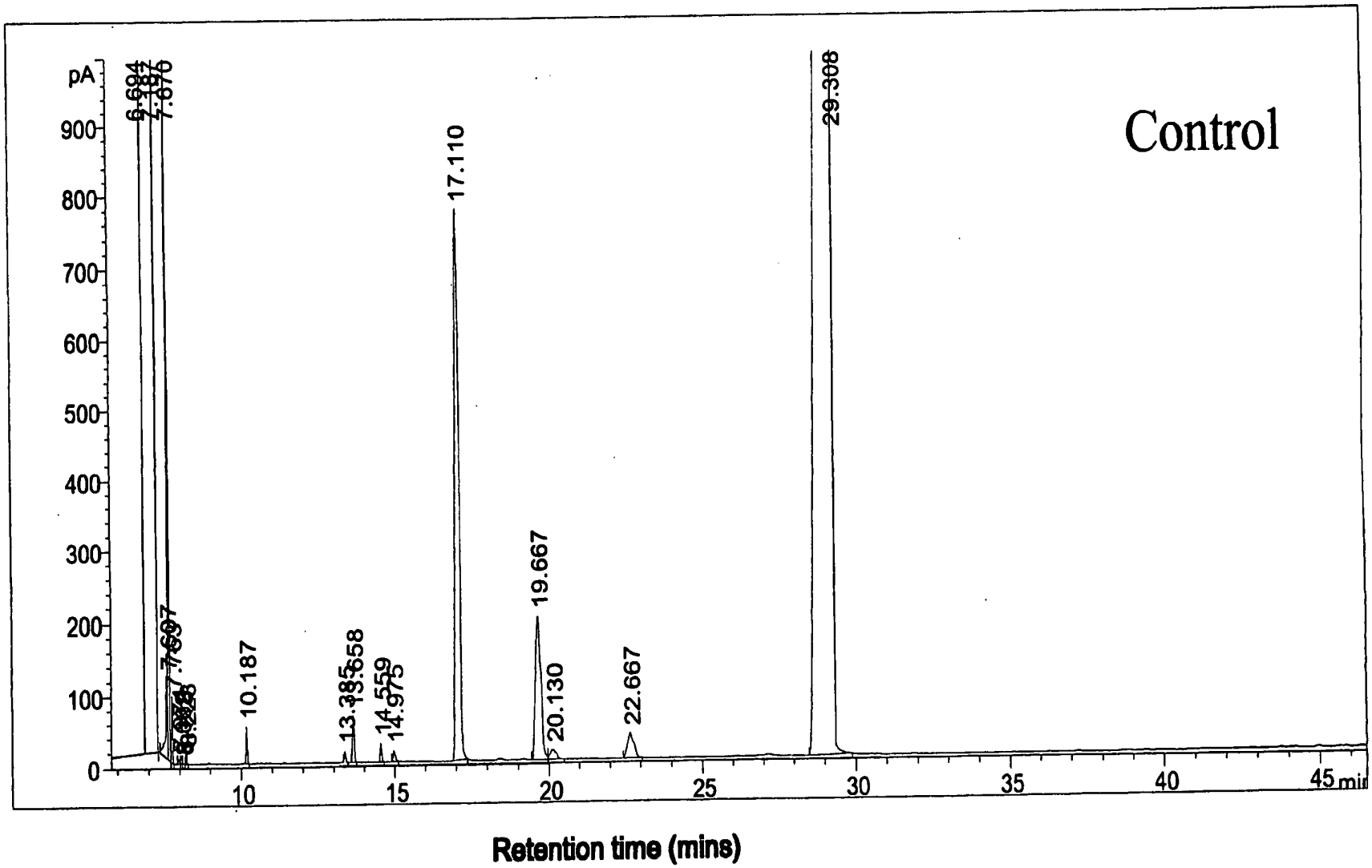
Control
pA
900
800
700
600
500
400
300
200
100
0
10.187
17.110
19.667
20.130
22.667
29.308
10
15
20
25
30
35
40
45 min
Retention time (mins)

FIG. 13A

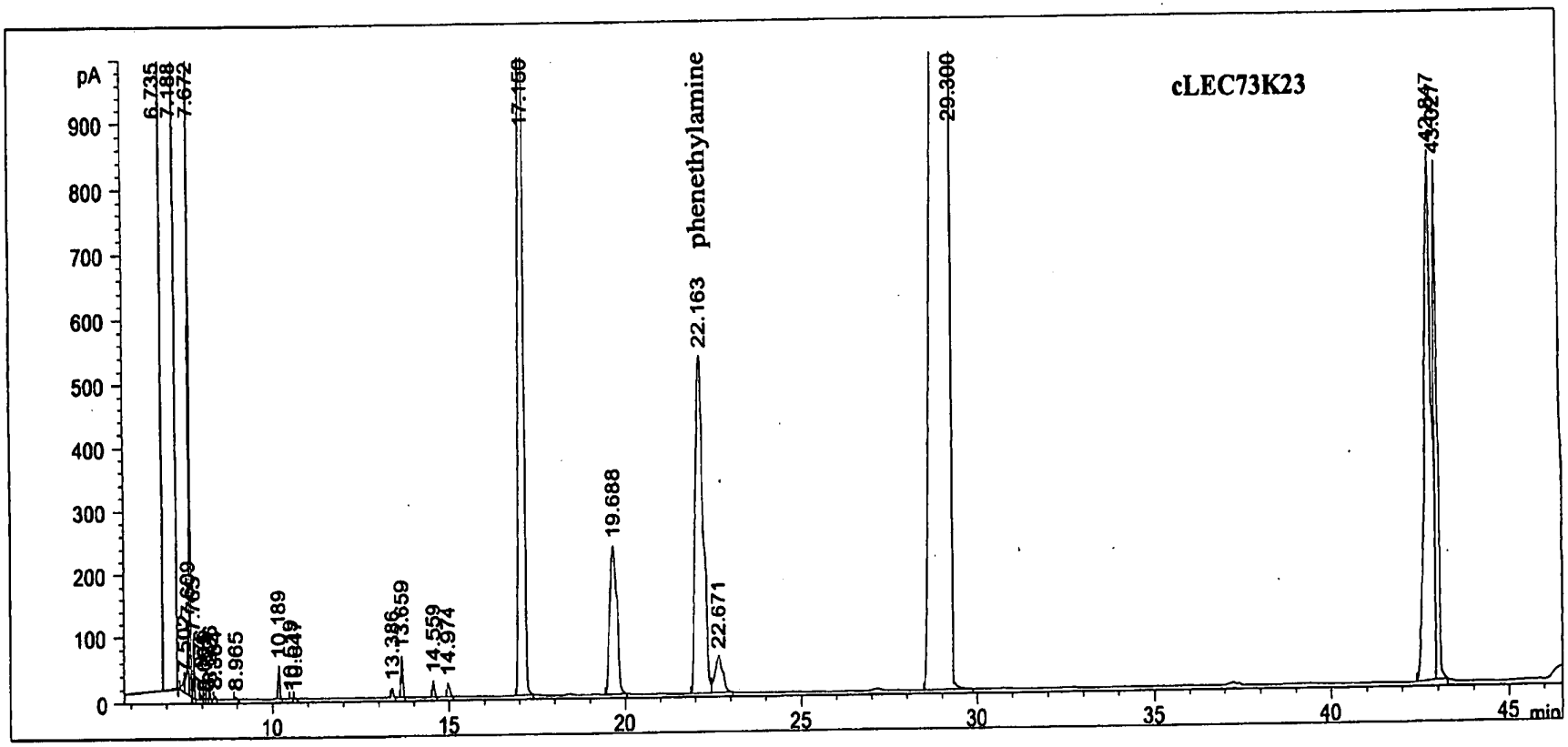
pA
900
800
700
600
500
400
300
200
100
0
6.735
7.188
7.672
8.965
10.189
13.386
13.659
14.559
14.974
17.166
19.688
22.163
phenethylamine
22.671
29.300
cLEC73K23
10
15
20
25
30
35
40
45 min
Retention time (mins)

FIG. 13B

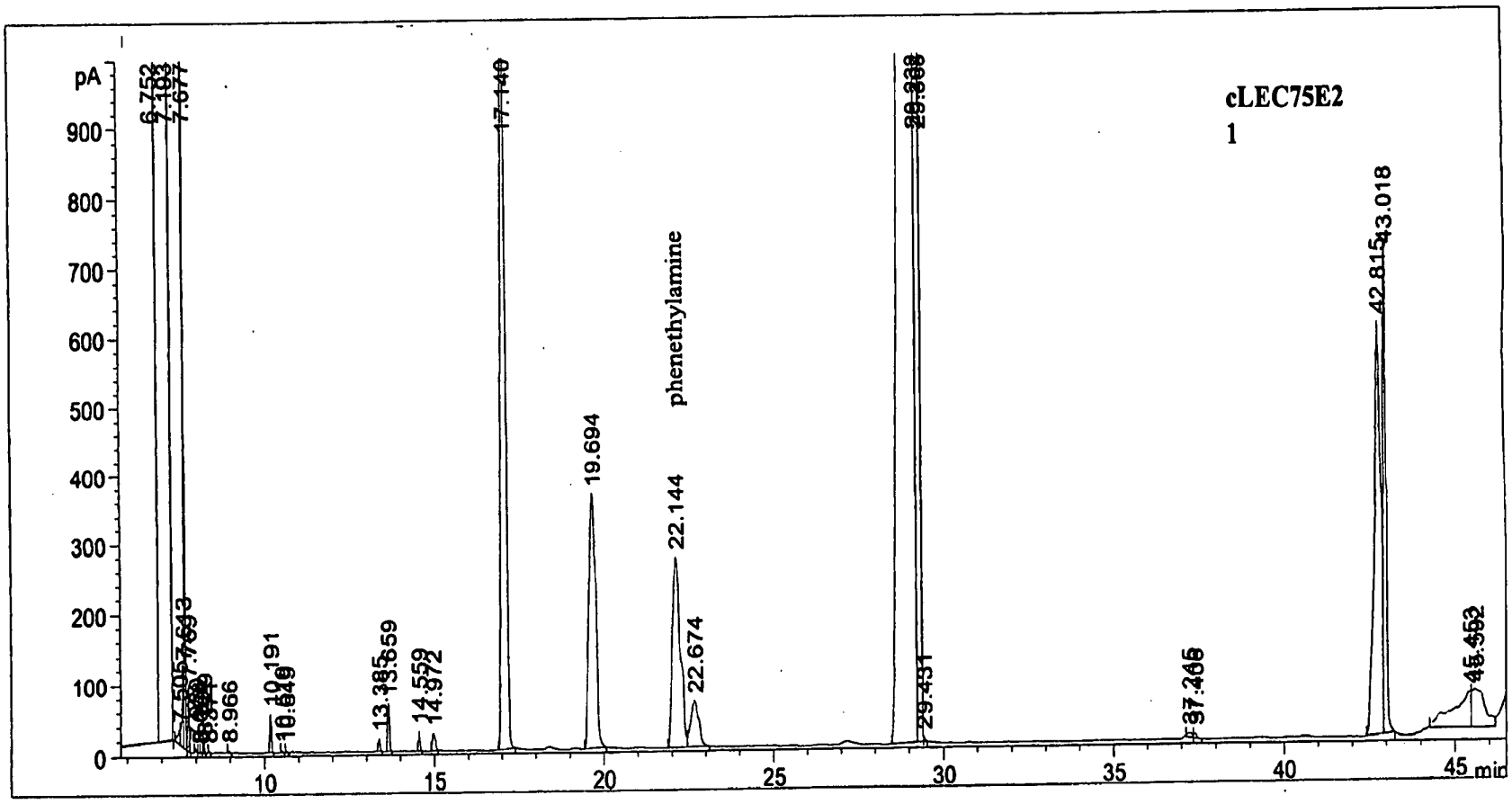
pA
900
800
700
600
500
400
300
200
100
0
cLEC75E2
1
19.694
phenethylamine
22.144
22.674
42.815
43.018
8.966
13.385
13.559
14.559
14.972
29.431
37.466
10
15
20
25
30
35
40
45 min
Retention time (mins)

FIG. 13C

SEQUENCE LISTING

<110> Klee, Harry J.
Tieman, Denise

<120> Isolation of a Gene Involved in Synthesis of a Flavor and Aroma Volatile in Plants

<130> UF-386CP

<160> 9

<170> PatentIn version 3.2

<210> 1
<211> 1367
<212> DNA
<213> Lycopersicon esculentum

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aaggataatc tctcaaatta ctttcttttt ttttcctatc aattctttat accaaaataa 120

tattattgtt tttttctcct ctgtttctgc ttcgtatttt tgctgagaga aatgagtgtg 180

acagcgaaaa cagtgtgtgt aacaggagct tcaggttaca tagcttcatg gctagtcaaa 240

ttcttgctac atagtggtta caatgtgaag gcttctgttc gtgatccaaa tgatcccaag 300

aaaacgcagc acttgctttc tcttggtggg gccaaggaga ggcttcactt gttcaaagca 360

aacctattag aagaaggttc atttgatgct gtagttgatg gatgtgaagg tgtattccat 420

acagcgtctc ctttttacta ctctgttaca gacccacagg ctgaattact tgatcctgct 480

gttaagggaa cactcaatct tctcgggtca tgtgccaaag caccatcagt aaaacgagtt 540

gttttaacgt cttccatagc tgcagttgct tacagtggtc agcctcggac acctgaggtt 600

gtggttgatg agagctggtg gaccagtcca gactactgca aagaaaaaca gctctggtat 660

gtcctctcaa agacattggc tgaggatgct gcgtggaagt ttgtgaagga gaaaggcatt 720

gatatggttg tagtaaaccc tgctatggtt attggtcctc tgttacagcc tacacttaat 780

accagttctg ctgcagtctt gagcttggta aatggtgctg agacataccc aaattcctct 840

tttgggtggg ttaacgtgaa agatgttgca aatgcacata ttcttgcatt tgagaaccct 900

tcagctaatg ggagatactt aatggttgag agggttgcac actattctga tatattgaag 960

atattgcgtg acctttatcc tactatgcaa cttccagaaa agtgtgctga tgacaaccca 1020

ttgatgcaaa attatcaagt atcaaaggag aaggcaaaaa gcttgggtat tgagtttact 1080

acccttgaag aaagcatcaa agaaactgtt gaaagtttga aggaaaagaa gttttttgga 1140

ggttcatctt ctatgtaaaa ggcttctcaa agcttttatg gttttgttga acaatactac 1200

ccaccccacc ctaccctaca cacttttttt ttttacttct tttagctaat tatagaatca 1260

agaagtcgaa tggtatatcc gttaataaat ttcgatcaga tgaggttgaa atttgttcta 1320

tatctagaga tttttacaga ctggtttgat agaaaaaaaa aaaaaaa 1367

<210> 2
<211> 328
<212> PRT
<213> Lycopersicon esculentum

<400> 2

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20 25 30

Lys Ala Ser Val Arg Asp Pro Asn Asp Pro Lys Lys Thr Gln His Leu
35 40 45

Leu Ser Leu Gly Gly Ala Lys Glu Arg Leu His Leu Phe Lys Ala Asn
50 55 60

Leu Leu Glu Glu Gly Ser Phe Asp Ala Val Val Asp Gly Cys Glu Gly
65 70 75 80

Val Phe His Thr Ala Ser Pro Phe Tyr Tyr Ser Val Thr Asp Pro Gln
85 90 95

Ala Glu Leu Leu Asp Pro Ala Val Lys Gly Thr Leu Asn Leu Leu Gly
100 105 110

Ser Cys Ala Lys Ala Pro Ser Val Lys Arg Val Val Leu Thr Ser Ser
115 120 125

Ile Ala Ala Val Ala Tyr Ser Gly Gln Pro Arg Thr Pro Glu Val Val
130 135 140

Val Asp Glu Ser Trp Trp Thr Ser Pro Asp Tyr Cys Lys Glu Lys Gln
145 150 155 160

Leu Trp Tyr Val Leu Ser Lys Thr Leu Ala Glu Asp Ala Ala Trp Lys
165 170 175

Phe Val Lys Glu Lys Gly Ile Asp Met Val Val Val Asn Pro Ala Met
180 185 190

```
Val Ile Gly Pro Leu Leu Gln Pro Thr Leu Asn Thr Ser Ser Ala Ala
     195                 200                 205

Val Leu Ser Leu Val Asn Gly Ala Glu Thr Tyr Pro Asn Ser Ser Phe
 210                 215                 220

Gly Trp Val Asn Val Lys Asp Val Ala Asn Ala His Ile Leu Ala Phe
225                 230                 235                 240

Glu Asn Pro Ser Ala Asn Gly Arg Tyr Leu Met Val Glu Arg Val Ala
                245                 250                 255

His Tyr Ser Asp Ile Leu Lys Ile Leu Arg Asp Leu Tyr Pro Thr Met
            260                 265                 270

Gln Leu Pro Glu Lys Cys Ala Asp Asp Asn Pro Leu Met Gln Asn Tyr
        275                 280                 285

Gln Val Ser Lys Glu Lys Ala Lys Ser Leu Gly Ile Glu Phe Thr Thr
    290                 295                 300

Leu Glu Glu Ser Ile Lys Glu Thr Val Glu Ser Leu Lys Glu Lys Lys
305                 310                 315                 320

Phe Phe Gly Gly Ser Ser Ser Met
                325
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<213> Artificial sequence

<220>
<223> PCR primer

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<211> 1398
<212> DNA
<213> Lycopersicon esculentum

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tcaacaccaa caacaccaag aaaaaacttg aatctttcag tgacggagcc aggaaaaaat 180

gatggaccta gtttggattg tacattgatg aattatattg atacactcac ccaacgtatc 240

aactatcata tcggttatcc agttaacata tgttatgagc actatgctaa tttagcccca 300

cttttacaat ttcatttaaa taattgtggt gatccatttc ttcaaaatac tgtggatttt 360

cattcaaagg attttgaagt ggctgtttta aattggtttg ctgatttatg ggaaattgaa 420

agagatcaat attggggcta tgtaacaaat ggtggtactg aaggaaattt acatggcatt 480

ttggttggga gagaattgtt tccagatgga attttatatg catcaaaaga ctctcattac 540

tcagtggcta aggcagcaat gatgtataga atggattttg aaaatattaa cgcatcaata 600

aatggagaaa tcgattattc tgatttgaaa gttaaattac ttcaaaacaa gggaaaacca 660

gcgataatta atgttacaat tggcactact tttaaaggag ctgttgatga tcttgatgtt 720

attcttcaaa tacttgaaga gtgtggttac acacgagatc aattttatat tcattgtgat 780

gcagcactaa atggacttat tattcctttt attaaaaata tgattacttt caagaagcca 840

attggaagtg tgacaatttc tggtcacaag tttttgggat gtccaatgcc ttgtggagtt 900

caaataacaa ggaaaagtta cattaataac ctttcgagaa gagtcgaata tattgcttct 960

gtggatgcta caatttctgg aagtcgaaat ggtttgactc cgatcttctt atggtacagt 1020

ataagtgcta aaggtcaaat tggttttcag aaagacgtta agagatgttt tgacaatgct 1080

aagtacttga aagaccgtct tcagcaagca ggaatcagcg tcatgctgaa tgagcttagc 1140

atcatagttg tcctcgagag gcctcgtgac catgaattcg ttcgtcgttg gcaattatct 1200

tgtgtgagag atatggcaca tgttattgtt atgccaggca taactagaga aactcttgat 1260

ggttttatta atgatttgct tcaacaaagg aaaaaatggt atcaagatgg aagaattagc 1320

cctccttgtg ttgcaaatga tattggtgct caaaattgtg cttgctctta tcataaaatt 1380

gattacatta ttgcttag 1398

<210> 5
<211> 465
<212> PRT
<213> Lycopersicon esculentum

<400> 5

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1 5 10 15

Pro Arg Gly Leu Ala Pro Pro Gly Leu Ile Val Asn Gly Asp Phe Gly
20 25 30

Glu Met Met Arg Leu Lys Val Ser Ser Thr Pro Thr Thr Pro Arg Lys
35 40 45

```
Asn Leu Asn Leu Ser Val Thr Glu Pro Gly Lys Asn Asp Gly Pro Ser
    50                  55                  60

Leu Asp Cys Thr Leu Met Asn Tyr Ile Asp Thr Leu Thr Gln Arg Ile
65                  70                  75                  80

Asn Tyr His Ile Gly Tyr Pro Val Asn Ile Cys Tyr Glu His Tyr Ala
                85                  90                  95

Asn Leu Ala Pro Leu Leu Gln Phe His Leu Asn Asn Cys Gly Asp Pro
            100                 105                 110

Phe Leu Gln Asn Thr Val Asp Phe His Ser Lys Asp Phe Glu Val Ala
        115                 120                 125

Val Leu Asn Trp Phe Ala Asp Leu Trp Glu Ile Glu Arg Asp Gln Tyr
    130                 135                 140

Trp Gly Tyr Val Thr Asn Gly Gly Thr Glu Gly Asn Leu His Gly Ile
145                 150                 155                 160

Leu Val Gly Arg Glu Leu Phe Pro Asp Gly Ile Leu Tyr Ala Ser Lys
                165                 170                 175

Asp Ser His Tyr Ser Val Ala Lys Ala Ala Met Met Tyr Arg Met Asp
            180                 185                 190

Phe Glu Asn Ile Asn Ala Ser Ile Asn Gly Glu Ile Asp Tyr Ser Asp
        195                 200                 205

Leu Lys Val Lys Leu Leu Gln Asn Lys Gly Lys Pro Ala Ile Ile Asn
    210                 215                 220

Val Thr Ile Gly Thr Thr Phe Lys Gly Ala Val Asp Asp Leu Asp Val
225                 230                 235                 240

Ile Leu Gln Ile Leu Glu Glu Cys Gly Tyr Thr Arg Asp Gln Phe Tyr
                245                 250                 255

Ile His Cys Asp Ala Ala Leu Asn Gly Leu Ile Ile Pro Phe Ile Lys
            260                 265                 270

Asn Met Ile Thr Phe Lys Lys Pro Ile Gly Ser Val Thr Ile Ser Gly
        275                 280                 285

His Lys Phe Leu Gly Cys Pro Met Pro Cys Gly Val Gln Ile Thr Arg
    290                 295                 300

Lys Ser Tyr Ile Asn Asn Leu Ser Arg Arg Val Glu Tyr Ile Ala Ser
305                 310                 315                 320

Val Asp Ala Thr Ile Ser Gly Ser Arg Asn Gly Leu Thr Pro Ile Phe
                325                 330                 335
```

Leu Trp Tyr Ser Ile Ser Ala Lys Gly Gln Ile Gly Phe Gln Lys Asp
340 345 350

Val Lys Arg Cys Phe Asp Asn Ala Lys Tyr Leu Lys Asp Arg Leu Gln
355 360 365

Gln Ala Gly Ile Ser Val Met Leu Asn Glu Leu Ser Ile Ile Val Val
370 375 380

Leu Glu Arg Pro Arg Asp His Glu Phe Val Arg Arg Trp Gln Leu Ser
385 390 395 400

Cys Val Arg Asp Met Ala His Val Ile Val Met Pro Gly Ile Thr Arg
405 410 415

Glu Thr Leu Asp Gly Phe Ile Asn Asp Leu Leu Gln Gln Arg Lys Lys
420 425 430

Trp Tyr Gln Asp Gly Arg Ile Ser Pro Pro Cys Val Ala Asn Asp Ile
435 440 445

Gly Ala Gln Asn Cys Ala Cys Ser Tyr His Lys Ile Asp Tyr Ile Ile
450 455 460

Ala
465

<210> 6
<211> 1416
<212> DNA
<213> Lycopersicon esculentum

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gcagcgatga cacctagaag tttagcgcga cgacgattgt ttccgaacgt ggacaacaag 120

aaacagaaaa tggcacaacc aggtgcagga ccaaggaaga acttggaact tgaggtcatg 180

gagcctgcat tgaagaatga tggtccttct ttggacacta tcttggttaa ttatttggac 240

acacttacac aacgagtcaa ttatcattta ggttatccag tcaacatatg ttatgatcac 300

tatgcaacgc tagcaccact tttgcagttt cacctaaaca attgtggtga tcctttccta 360

caaaatactg tcgatttcca ttctaaagac tttgaagtgg ctgttttgaa ttggtttgca 420

aaactttggg aaattgaaaa ggatcaatat tggggatatg ttaccaatgg tggcaccgaa 480

ggcaatctcc atggtatttt gttagggaga gagctacttc ctgaaggaat attatatgca 540

tcaaaagact ctcattactc agtattcaaa gctgcaagaa tgtatagaat ggattcagaa 600

acaatcaaca catcagtaaa tggagagatg gattattcag atttaagagc aaagttactt 660

caaaataagg ataaaccagc tattataaat gtcacaattg gaactacatt caaaggagca 720

atcgatgacc tggatgttat tcttgaaata ctcaaagaat gtggctattc acaagatcga 780

ttttacattc actgtgatgc agcactatgt ggtcttatga ccccttttat aaacaatatg 840

attagtttca agaagccaat tggaagtgtc acaatttctg gacacaagtt tttgggatgt 900

ccaatgcctt gtggtgtcca aataacaaga aaaagctaca tcaataatct ctcaacaaat 960

gtggaataca ttgcttctgt ggatgccact atttctggta gccgtaacgg tttaactcca 1020

attttcttat ggtatagctt gagcgcaaaa ggtcaagttg gacttcaaaa ggatgttaaa 1080

agatgtctcg acaatgccaa atatttgaaa gatcgtcttc aacaagcagg gataagtgtc 1140

atgctgaatg agctaagcat catagttgta cttgaaaggc ctcgtgacca tgaatttgtg 1200

cgtcgttggc aactctcatg cgtcaaggat atggcacatg ttattgtgat gccaggaatc 1260

acacgagaaa tgcttgacaa cttcatgagt gaattagtgc aacaaagaaa agtatggtat 1320

caaaatggaa agactgatcc tccttgtgtt ggagaggata ttggtgctca aaattgtgca 1380

tgctcttatc ataagattga ctacatctgt ccttag 1416

<210> 7
<211> 471
<212> PRT
<213> Lycopersicon esculentum

<400> 7

Met Gly Ser Leu Ser Leu Glu Met Asp Phe Glu Pro Ser Pro Met Thr
1 5 10 15

Pro Arg Ser Leu Ala Ala Met Thr Pro Arg Ser Leu Ala Arg Arg Arg
20 25 30

Leu Phe Pro Asn Val Asp Asn Lys Lys Gln Lys Met Ala Gln Pro Gly
35 40 45

Ala Gly Pro Arg Lys Asn Leu Glu Leu Glu Val Met Glu Pro Ala Leu
50 55 60

Lys Asn Asp Gly Pro Ser Leu Asp Thr Ile Leu Val Asn Tyr Leu Asp
65 70 75 80

Thr Leu Thr Gln Arg Val Asn Tyr His Leu Gly Tyr Pro Val Asn Ile
85 90 95

Cys Tyr Asp His Tyr Ala Thr Leu Ala Pro Leu Leu Gln Phe His Leu
100 105 110

```
Asn Asn Cys Gly Asp Pro Phe Leu Gln Asn Thr Val Asp Phe His Ser
        115                 120                 125

Lys Asp Phe Glu Val Ala Val Leu Asn Trp Phe Ala Lys Leu Trp Glu
    130                 135                 140

Ile Glu Lys Asp Gln Tyr Trp Gly Tyr Val Thr Asn Gly Gly Thr Glu
145                 150                 155                 160

Gly Asn Leu His Gly Ile Leu Leu Gly Arg Glu Leu Leu Pro Glu Gly
                165                 170                 175

Ile Leu Tyr Ala Ser Lys Asp Ser His Tyr Ser Val Phe Lys Ala Ala
            180                 185                 190

Arg Met Tyr Arg Met Asp Ser Glu Thr Ile Asn Thr Ser Val Asn Gly
        195                 200                 205

Glu Met Asp Tyr Ser Asp Leu Arg Ala Lys Leu Leu Gln Asn Lys Asp
    210                 215                 220

Lys Pro Ala Ile Ile Asn Val Thr Ile Gly Thr Thr Phe Lys Gly Ala
225                 230                 235                 240

Ile Asp Asp Leu Asp Val Ile Leu Glu Ile Leu Lys Glu Cys Gly Tyr
                245                 250                 255

Ser Gln Asp Arg Phe Tyr Ile His Cys Asp Ala Ala Leu Cys Gly Leu
            260                 265                 270

Met Thr Pro Phe Ile Asn Asn Met Ile Ser Phe Lys Lys Pro Ile Gly
        275                 280                 285

Ser Val Thr Ile Ser Gly His Lys Phe Leu Gly Cys Pro Met Pro Cys
    290                 295                 300

Gly Val Gln Ile Thr Arg Lys Ser Tyr Ile Asn Asn Leu Ser Thr Asn
305                 310                 315                 320

Val Glu Tyr Ile Ala Ser Val Asp Ala Thr Ile Ser Gly Ser Arg Asn
                325                 330                 335

Gly Leu Thr Pro Ile Phe Leu Trp Tyr Ser Leu Ser Ala Lys Gly Gln
            340                 345                 350

Val Gly Leu Gln Lys Asp Val Lys Arg Cys Leu Asp Asn Ala Lys Tyr
        355                 360                 365

Leu Lys Asp Arg Leu Gln Gln Ala Gly Ile Ser Val Met Leu Asn Glu
    370                 375                 380

Leu Ser Ile Ile Val Val Leu Glu Arg Pro Arg Asp His Glu Phe Val
385                 390                 395                 400
```

```
Arg Arg Trp Gln Leu Ser Cys Val Lys Asp Met Ala His Val Ile Val
                405                 410                 415

Met Pro Gly Ile Thr Arg Glu Met Leu Asp Asn Phe Met Ser Glu Leu
            420                 425                 430

Val Gln Gln Arg Lys Val Trp Tyr Gln Asn Gly Lys Thr Asp Pro Pro
        435                 440                 445

Cys Val Gly Glu Asp Ile Gly Ala Gln Asn Cys Ala Cys Ser Tyr His
    450                 455                 460

Lys Ile Asp Tyr Ile Cys Pro
465                 470
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<210> 8
<211> 1416
<212> DNA
<213> Lycopersicon esculentum

<400> 8

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aaacaaaagg tgcaacaatc aggtgcaggg ccaaggaaga acttacaact tgaagtcatg    180

gaacctgcat tgaacaatgc tggtccctct ttggacacta tattggtcaa ttatttagac    240

acacttacac aacgagtcaa ttatcattta ggttatccag tcaacatttg ttatgatcac    300

tatgcaactt tagcaccact tttacagttt cacctaaaca attgtggtga tcctttccta    360

caaaacactg tcgatttcca ttctaaagac tttgaagtgg ctgttttgaa ttggtttgca    420

aaactatggg aaattgaaaa ggatcaatac tggggatatg ttaccaatgg tggcaccgaa    480

ggcaatctcc atggtatttt gttagggaga gagctacttc ctgatggaat attatatgcg    540

tcaaaagact ctcactattc ggtcttcaaa gctgcaagaa tgtatagaat ggattcagaa    600

acaatcaaca catcagtaaa cggagagatg gattattcag atttaagagc aaagttactt    660

caaaataagg ataaaccagc tattataaat gtcacaattg gaactacgtt caaaggagca    720

atcgatgacc tggatgttat tcttgaaaca ctcaaagaat gtggctattc gcaagatagg    780

ttttacatcc actgtgatgc tgcactatgt ggtcttatga ccccttttat aaacaatatg    840

attagtttca agaagccaat tggaagtgtc acaatttctg gacacaagtt tttgggatgt    900

ccaatgcctt gtggtgtcca aattacaaga aagagttaca tcaataatct ctcaacaaat    960
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gtggaataca ttgcttctgt cgatgccact atttctggca gccgtaacgg tttaactcca 1020

attttcttgt ggtatagctt gagcgcaaaa ggtcaagttg gacttcaaaa ggatgttaaa 1080

agatgtctcg acaatgccaa atatttgaaa gatcgtcttc aaaaagcagg aataagtgtc 1140

atgttaaatg agcttagcat catagttgta cttgaaaggc ctcgtgacca tgaatttgtc 1200

cgtcgttggc aactctcatg cgtcaaggat atggcacatg ttattgtaat gccaggcatc 1260

acacgagaaa tgcttgacaa tttcacgagt gaattagtgc aacaaagaaa agtatggtat 1320

caaaatggac agaccaatcc tccttgtgtt ggagaggata ttggtgctca aaattgtgca 1380

tgctcttatc ataagattga ctacatctgt ccttag 1416

<210> 9
<211> 471
<212> PRT
<213> Lycopersicon esculentum

<400> 9

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1 5 10 15

Pro Arg Ser Leu Ala Ala Met Thr Pro Arg Ser Leu Ala Arg Arg Arg
20 25 30

Leu Phe Pro Asn Val Asp Asn Lys Lys Gln Lys Val Gln Gln Ser Gly
35 40 45

Ala Gly Pro Arg Lys Asn Leu Gln Leu Glu Val Met Glu Pro Ala Leu
50 55 60

Asn Asn Ala Gly Pro Ser Leu Asp Thr Ile Leu Val Asn Tyr Leu Asp
65 70 75 80

Thr Leu Thr Gln Arg Val Asn Tyr His Leu Gly Tyr Pro Val Asn Ile
85 90 95

Cys Tyr Asp His Tyr Ala Thr Leu Ala Pro Leu Leu Gln Phe His Leu
100 105 110

Asn Asn Cys Gly Asp Pro Phe Leu Gln Asn Thr Val Asp Phe His Ser
115 120 125

Lys Asp Phe Glu Val Ala Val Leu Asn Trp Phe Ala Lys Leu Trp Glu
130 135 140

Ile Glu Lys Asp Gln Tyr Trp Gly Tyr Val Thr Asn Gly Gly Thr Glu
145 150 155 160

Gly Asn Leu His Gly Ile Leu Leu Gly Arg Glu Leu Leu Pro Asp Gly

```
                165                 170                 175

Ile Leu Tyr Ala Ser Lys Asp Ser His Tyr Ser Val Phe Lys Ala Ala
            180                 185                 190

Arg Met Tyr Arg Met Asp Ser Glu Thr Ile Asn Thr Ser Val Asn Gly
        195                 200                 205

Glu Met Asp Tyr Ser Asp Leu Arg Ala Lys Leu Leu Gln Asn Lys Asp
    210                 215                 220

Lys Pro Ala Ile Ile Asn Val Thr Ile Gly Thr Thr Phe Lys Gly Ala
225                 230                 235                 240

Ile Asp Asp Leu Asp Val Ile Leu Glu Thr Leu Lys Glu Cys Gly Tyr
                245                 250                 255

Ser Gln Asp Arg Phe Tyr Ile His Cys Asp Ala Ala Leu Cys Gly Leu
            260                 265                 270

Met Thr Pro Phe Ile Asn Asn Met Ile Ser Phe Lys Lys Pro Ile Gly
        275                 280                 285

Ser Val Thr Ile Ser Gly His Lys Phe Leu Gly Cys Pro Met Pro Cys
    290                 295                 300

Gly Val Gln Ile Thr Arg Lys Ser Tyr Ile Asn Asn Leu Ser Thr Asn
305                 310                 315                 320

Val Glu Tyr Ile Ala Ser Val Asp Ala Thr Ile Ser Gly Ser Arg Asn
                325                 330                 335

Gly Leu Thr Pro Ile Phe Leu Trp Tyr Ser Leu Ser Ala Lys Gly Gln
            340                 345                 350

Val Gly Leu Gln Lys Asp Val Lys Arg Cys Leu Asp Asn Ala Lys Tyr
        355                 360                 365

Leu Lys Asp Arg Leu Gln Lys Ala Gly Ile Ser Val Met Leu Asn Glu
    370                 375                 380

Leu Ser Ile Ile Val Val Leu Glu Arg Pro Arg Asp His Glu Phe Val
385                 390                 395                 400

Arg Arg Trp Gln Leu Ser Cys Val Lys Asp Met Ala His Val Ile Val
                405                 410                 415

Met Pro Gly Ile Thr Arg Glu Met Leu Asp Asn Phe Thr Ser Glu Leu
            420                 425                 430

Val Gln Gln Arg Lys Val Trp Tyr Gln Asn Gly Gln Thr Asn Pro Pro
        435                 440                 445

Cys Val Gly Glu Asp Ile Gly Ala Gln Asn Cys Ala Cys Ser Tyr His
    450                 455                 460
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Lys Ile Asp Tyr Ile Cys Pro
465                 470
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DESCRIPTION

MATERIALS AND METHODS FOR SYNTHESIS OF A FLAVOR AND AROMA VOLATILE IN PLANTS

This invention was made with government support under National Science Foundation grant number 7223426-12. The government has certain rights in the invention.

Background of the Invention

Fruits are major components of the human diet contributing a large portion of vitamins, minerals, antioxidants, and fiber. While flavor and nutrition composition have clear and profound potential for positive human benefit, they have proven to be difficult traits to modify via either traditional breeding or transgenic approaches due to their generally complex biosynthetic and regulatory pathways. In fact, the biochemical descriptors that comprise flavor are poorly defined. What is typically perceived as flavor in many fruits is the product of a complex interaction among sugars, acids and multiple volatile secondary metabolites (Buttery *et al.*, 1988; Baldwin *et al.*, 2000). Synthesis and accumulation of these compounds is the result of coordinated activity of many genes that may also impact additional aspects of plant growth and development. Effective manipulation of these traits for human benefit will therefore require greater knowledge of the pathways involved and the regulatory systems which control them. Prior to the advent of genomics, researchers could focus on the activity of only one to several genes important in a process of interest and could view their respective effects in relative isolation. From a practical perspective, flavor and nutrition are intimately related and equally important as flavor directly impacts the choice of foods for consumption which, in turn, has positive nutritional consequences on the human diet.

Fruit-bearing crop plants are taxonomically diverse (*e.g.*, pepper, tomato, melons, apples, bananas, grapes). However, they do share common features; most, though not all, fruits are enlarged ovaries. While our knowledge of how domesticated plants came to bear fruit or the mechanisms by which they ripen is still rudimentary, more is known about these processes in tomato (*Lycopersicon esculentum*) than in any other species (see Giovannoni (2001) for review).

Furthermore, a diverse set of Near Isogenic Lines (NILs), single gene ripening mutants, and transgenic lines represent portals through which genetic regulation of fruit development and ripening can be studied (Gray *et al.*, 1994; Giovannoni *et al.*, 1999). The diversity of genetically well characterized tomato germplasm described below (Table 1) is unparalleled in other fruiting species. Nevertheless, it is important to realize that while fruit ripening is a complex sum of coordinately regulated biochemical events that vary from species to species, key regulatory components are likely to be maintained (Hobson and Grierson, 1993). For example, one group has recently identified two genes that are essential for fruit ripening, RIN and NOR (Giovannoni, 2001; Vrebalov *et al.*, 2002). Fruit-specific, ripening-induced homologues of these genes have been identified from strawberry and banana (Vrebalov *et al.*, 2002). Strawberry undergoes a very different ripening program as compared to tomato in that strawberry is non-climacteric (*i.e.* no increase in respiration or ethylene biosynthesis during ripening) and accumulates high levels of anthocyanins rather than carotenoids during fruit maturation. Further, it is anatomically a receptacle, whereas most fruits are ovaries. Banana is interesting in that while its fruit are also expanded carpels, it is a monocot. Apparently similar ripening control shared among monocots and dicots indicates that basic ripening regulation is likely conserved through evolution. In summary, these results suggest that while specific nutritional and flavor components may vary among fruit species they are likely due to regulated metabolic flux through similar pathways with similar genetic control systems. Thus, regulatory and biosynthetic genes identified in tomato will allow for modification of the same or related compounds in a wide range of agriculturally important fruit species.

Tomato has long served as a model system for plant genetics, development, physiology and fruit ripening resulting in the accumulation of substantial information regarding the biology of this economically important plant. Many experimental tools and features of tomato make it ideal for study of fruit ripening; these include extensive germplasm collections, numerous natural, induced, and transgenic mutants, routine transformation technology, a dense and expanding RFLP map, numerous cDNA and genomic libraries, a small genome, relatively short life-cycle and ease of growth and maintenance. In addition, numerous genomic tools that have and continue to be developed include: a) over 140,000 EST sequences (~30,000 non-redundant) from 23 different tomato tissues/treatments (with one-third of the ESTs derived from fruit), b)

EST expression arrays being developed and utilized (see bti.cornell.edu/CGEP/CGEP.html) and c) recent initiation of activities toward development of a tomato physical map anchored to the genetic map to facilitate gene isolation and eventual genome sequencing (Tanksley *et al.*, NSF tomato genome project, 1992). The intense research effort in tomato fruit biology has resulted in many important discoveries that have had a broad impact on the field of plant biology, including control of gene expression by antisense technology, characterization of numerous genes influencing fruit development and ripening, characterization of genes for ethylene synthesis and perception, and the recent connection of ripening regulation and ethylene response to the molecular regulation of floral development (Vrebalov *et al.*, 2002).

Fruit maturation and ripening is the summation of biochemical and physiological changes occurring at the terminal stage of development rendering the organ edible and valuable as an agricultural commodity. These changes frequently include modification of cell wall ultrastructure and texture, conversion of starch to sugars, alterations in pigment and nutrient biosynthesis/accumulation, and heightened levels of flavor and aromatic volatiles (Rhodes, 1980; Hobson and Grierson, 1993). While some ripening effects, such as carotenoid and vitamin C synthesis and accumulation, have direct impact on the nutritive value of mature fruit, others impacting flavor and texture (*e.g.*, volatiles, sugars and acids) can have an indirect impact on human nutrition via their contributions to total consumption levels. In short, "if it tastes better" consumption will increase. This is especially critical as poor food choices exert a disproportional impact on children and members of society on lower rungs of the socio-economic ladder.

Although most fruits display modifications in color, texture, flavor and nutrient composition during maturation, two major classifications of ripening, climacteric and non-climacteric, have been utilized to distinguish fruit on the basis of respiration and ethylene synthesis rates. Climacteric fruits such as tomato, avocado, banana, peaches and apples, are distinguished from non-climacteric fruits such as strawberry, grape and citrus, by their increased respiration and ethylene synthesis rates during ripening (Lelievre *et al.*, 1998). In tomato, ethylene has been shown to be necessary for the coordination and completion of ripening (Yang, 1985; Tucker and Brady, 1987; Klee *et al.*, 1991; Picton *et al.*, 1993; Lanahan *et al.*, 1994). The critical role of ethylene in coordinating climacteric ripening at the molecular level was first

observed via analysis of ethylene inducible ripening-related gene expression in tomato (Lincoln *et al.*, 1987; Maunders *et al.*, 1987). Numerous fruit development-related genes have since been isolated via differential expression patterns and biochemical function (reviewed in Gray *et al.*, 1994). The *in vivo* functions of many fruit development- and ripening-related genes have been tested via antisense repression and/or mutant complementation in tomato. As examples, polygalacturonase was shown to be necessary for ripening-related pectin depolymerization and pathogen susceptibility, yet to have little effect on fruit softening (Smith *et al.*, 1988, Giovannoni *et al.*, 1989, Kramer *et al.*, 1990). Inhibition of phytoene synthase resulted in reduced carotenoid biosynthesis and reduction in fruit and flower pigmentation (Fray and Grierson, 1993). Reduced ethylene evolution resulted in ripening inhibition of ACC synthase (ACS) and ACC oxidase (ACO) antisense lines (Oeller *et al.*, 1991; Hamilton *et al.*, 1990) while introduction of a dominant mutant allele of the *NR* ethylene receptor resulted in plants inhibited in virtually every measurable ethylene response including fruit ripening (Wilkinson *et al.*, 1995; Yen *et al.*, 1995).

Expression analysis of multiple tomato ripening-related genes indicates that a subset exhibit developmentally-controlled ethylene inducibility, *i.e.*, they are ethylene inducible only in ripening fruits. Examples include members of the ACO and ACS gene families (Theologis *et al.*, 1993; Blume and Grierson, 1997; Nakatsuka *et al.*, 1998), the *NR* ethylene receptor (Wilkinson *et al.*, 1995; Payton *et al.*, 1996; Lashbrook *et al.*, 1998) and E8 (Deikman *et al.*, 1992). Additional evidence for non-ethylene mediated ripening control comes from analysis of gene expression in ripening impaired mutants such as *rin* (*ripening-inhibitor*) and *nor* (*non-ripening*) that fail to ripen in response to exogenous ethylene yet display signs of ethylene sensitivity and signaling including induction of some ethylene-regulated genes (Yen *et al.*, 1995). These results suggest that regulatory constraints are placed on climacteric fruit maturation in addition to general ethylene biosynthesis and signaling. Such mechanisms could include fruit-specific regulation of certain subsets of ethylene regulated genes or factors that operate separate from and in addition to ethylene as seems to be the case for both the RIN (Vrebalov *et al.*, 2002) and NOR transcription factors. This is particularly interesting as a greater understanding of the relationship between ethylene, developmental, and environmental signals will likely reveal the impact of various signaling systems on pathways impacting flavor and human nutrition. Indeed numerous environmental factors such as light and temperature can dramatically influence the

degree and rate of fruit ripening with significant impacts on the accumulation of carotenoids and flavor compounds (Hobson and Grierson, 1993; Yen *et al.*, 1997).

Numerous plant metabolites can be listed when the net of "nutritive compounds" is cast. These include various antioxidants, vitamins, minerals, fiber, lipids, and amino acids, to name just a few. In addition, as noted above, one can rationally argue that modification of flavor and additional quality attributes may lead to improved health via increased fruit or vegetable consumption.

Tomato fruits are among the highest source of lycopene, β-carotene, and vitamin C (ascorbate) in the diets of humans in the US, South America, and Europe, with steadily increasing prominence in Asia and the Middle East. In addition to direct nutritive value, carotenoids in particular are metabolized to compounds that impact flavor and aroma of fruit and thus have a significant impact on resulting fresh and processed products. Genes encoding the synthetic steps from phytoene through β-carotene (Bartley *et al.*, 1994; Ronen *et al.*, 1999) are potential regulatory points for modification of carotenoid levels. Indeed, available data indicate that accumulation of lycopene is due to coordinated up-regulation of the genes preceding its synthesis and down-regulation of genes that further metabolize it during ripening (Ronen *et al.*, 1999). Numerous mutant, transgenic, RI and breeding lines that display a wide range of levels of lycopene and β-carotene are available (Table 1). While specific mutants represent some of the catalytic steps (*e.g.*, *r* = phytoene synthase and *cr* and *B* = lycopene cyclase; Hamilton *et al.*, 1990; Ronen *et al.*, 1999) others such as *hp-1* and *hp-2* represent regulators of environmental response. Antisense phytoene synthase tomato lines are greatly reduced in all of the carotenoid-derived volatiles (Baldwin *et al.*, 2000). Furthermore, transgenic and mutant lines altered in ethylene synthesis or perception display variation in carotenoid levels (Table 1).

Table 1. Tomato germplasm altered in carotenoids, flavonoids, vitamin C.

Genotype	Carotenoids	Vit.C	Volatiles	Function
*rin; ripening-inhibitor**	very low	low	NA	MADS-box protein
*nor; non-ripening**	low	low	NA	transcription factor
*Nr: Never-ripe**	low	NA	NA	ethylene receptor
hp-2; high-pigment-2	high	high	NA	DET1 (light signaling)
cr; crimson	low B, high L	high	NA	lycopene cyclase
B; Beta	high B, low L	NA	NA	lycopene cyclase
r; Phytoene Synthase	low	NA	low	phytoene synthase
*hp-1; high-pigment-1***	high	high	NA	Not cloned (light signaling)
Nr-2: Never-ripe-2	low	NA	NA	Not cloned
Gr: Green-ripe	low	NA	NA	Not cloned
t; tangerine	low	NA	NA	Not cloned
at; apricot	low	NA	NA	Not cloned
Cnr; Clear non-ripening	low	NA	NA	Not cloned
L. esculentum x *L. pennellii* Recombinant Inbreds	low-high	low-high	low-high	
ACO; ACC oxidase*	low	NA	NA	ethylene Biosynthesis
ACS; ACC synthase*	low	NA	NA	ethylene Biosynthesis
ACD; ACC deaminase*	low	NA	NA	ethylene Biosynthesis
TCTR1; tomato CTR1*	low-high	NA	NA	ethylene signaling MAPKKK

The dashed line separates mutants for which the corresponding gene has been cloned (1st tier) from those which have not (2nd tier). The last tier indicates transgenic lines altered in ethylene synthesis or response and with corresponding changes in carotenoid accumulation. Genotypes indicated with an (*) represent those for which multiple independent transgenic lines are available demonstrating a range of carotenoid accumulation levels. **Three different mutant alleles of *hp-1* each having varying degrees of effect on carotenoid and flavonoid accumulation were provided by M. Koornneef. B = β-carotene. L = lycopene. While quantitative data for vitamin C and volatiles are unknown for many of these lines (NA), their respective phenotypes suggest they are likely to be altered in one or both.

In the case of flavor volatiles, the pathways for synthesis are in many cases not well established. For example, synthesis of apocarotenoids such as β-ionone and β-damascenone is not at all understood. Only recently has an Arabidopsis enzyme, CCD1 (Related to Carotenoid Dioxygenases), that synthesizes apocarotenoids such as β-ionone *in vitro* been identified

(Schwartz *et al.*, 2001). This gene is part of a multigene family, some of which are responsible for synthesis of other apocarotenoids such as ABA (Tan *et al.*, 1997). CCD1 cleaves multiple carotenoid substrates at the 9-10 and 9'-10' bonds, potentially releasing volatiles such as β-ionone, although this has not been established *in vivo*. Similarly, several different volatiles are derived from lipid breakdown (Table 2). The likely first step in their syntheses is the action of a lipoxygenase (LOX) (Riley and Thompson, 1997; Baldwin *et al.*, 2000). Currently there are 14 different EST contigs in the tomato database putatively identified as LOX. Any LOX exhibiting correlation with the lipid-derived volatiles would be a candidate sequence for analyses. It is exactly this sort of correlative biochemical and expression approach that resulted in identification of a key enzyme in strawberry volatile synthesis (Aharoni *et al.*, 2000).

Table 2. The 16 most significant flavor volatiles of tomato

Volatile	Conc. (ppb)	Log odor units	Precursor	Odor Characteristics
cis-3-Hexenal	12,000	3.7	lipid	tomato/green
β-ionone	4	2.8	carotenoid	fruity/floral
Hexanal	3,100	2.8	lipid	green/grassy
β-Damascenone	1	2.7	carotenoid	fruity
1-Penten-3-one	520	2.7	lipid	fruity floral/green
2+3-Methylbutanal	27	2.1	ILE/LEU	musty
trans-2-Hexenal	270	1.2	lipid	green
2-Isobutylthiazole	36	1.0	LEU	tomato vine
1-nitro-2-Phenylethane	17	0.9	PHE	musty, earthy
trans-2-Heptenal	60	0.7	lipid	green
Phenylacetaldehyde	15	0.6	PHE	floral/alcohol
6-Methyl-5-hepten-2-one	130	0.4	carotenoid	fruity, floral
cis-3-Hexenol	150	0.3	lipid	green
2-Phenylethanol	1,900	0.3	PHE	nutty
3-Methylbutanol	380	0.2	LEU	earthy, musty
Methyl salicylate	48	0.08	PHE	wintergreen

Volatiles are ranked by importance based on Odor Units (concentration X humans' ability to detect). Concentrations are average values from typical commercial tomatoes. Odor characteristics were determined by a trained expert panel.

Brief Summary of the Invention

The subject invention concerns polynucleotides encoding a plant 2-phenylethanol dehydrogenase enzyme. In one embodiment, the polynucleotide encodes a tomato 2-phenylethanol dehydrogenase. The subject invention also concerns 2-phenylethanol dehydrogenase polypeptides encoded by polynucleotides of the present invention.

The subject invention also concerns polynucleotides encoding a plant phenylalanine decarboxylase enzyme. In one embodiment, the polynucleotide encodes a tomato phenylalanine decarboxylase. The subject invention also concerns phenylalanine decarboxylase polypeptides encoded by polynucleotides of the present invention.

The subject invention also concerns methods for providing a plant with an increased flavor and aroma volatile. Plants can be transformed with one or more polynucleotides of the present invention. The subject invention also concerns these transformed plant cells, plant tissue, and plants and transgenic progeny thereof.

Brief Description of the Figures

Figures 1A and 1B show the results of GC profiles of volatiles collected from M82 or introgression line 8-2-1 ripe fruit. Introgression line 8-2-1 fruit have higher levels of phenylacetaldehyde and 2-phenylethanol than control M82 fruit.

Figure 2 is a graph of phenylacetaldehyde and 2-phenylethanol levels in control M82 and *L. pennellii* introgression line 8-2-1 fruit. Data are presented as % of control M82 fruit.

Figures 3A and 3B show a full-length cDNA (SEQ ID NO. 1) and amino acid sequence (SEQ ID NO. 2) of a 2-phenylethanol dehydrogenase of the present invention.

Figure 4 shows a pathway for production of the volatiles compounds phenylacetaldehyde and 2-phenylethanol in plants. Phenylalanine is decarboxylated by phenylalanine decarboxylase to form phenethylamine. Phenethylamine is then converted to phenylacetaldehyde by an amine oxidase, followed by conversion to 2-phenylethanol by 2-phenylethanol dehydrogenase.

Figures 5A, 5B, and 5C show the result of alcohol dehydrogenase activities of 2-phenylethanol dehydrogenase on phenylacetaldehyde and related substrates. Activity is determined by the disappearance of substrate and a reduction in OD (340nm). Highest activity levels are observed with phenylacetaldehyde as a subtrate.

Figures 6A and 6B show the result of GC profiles of volatiles emitted from wild-type Mitchell Diploid (MD) petunia flowers and transgenic petunia flowers expressing the tomato 2-phenylethanol dehydrogenase gene. In transgenic flowers, higher levels of 2-phenylethanol and lower levels of phenylacetaldehyde are observed as compared to wild-type flowers.

Figure 7 is a graph of the levels of 2-phenylethanol and phenylacetaldehyde emitted from petunia flowers of wild-type Mitchell Diploid (MD) and transgenic lines expressing the tomato 2-phenylethanol dehydrogenase gene.

Figures 8A and 8B show a full-length coding sequence (SEQ ID NO. 4) and amino acid sequence (SEQ ID NO. 5) of tomato phenylalanine decarboxylase cLEC75E21 of the present invention.

Figures 9A and 9B show a full-length coding sequence (SEQ ID NO. 6) and amino acid sequence (SEQ ID NO. 7) of tomato phenylalanine decarboxylase cLEC73K23 of the present invention.

Figure 10 is a Southern blot showing that cLEC73K23 is present on the *L. pennellii* introgression 8-2-1 on tomato chromosome 8. Restriction patterns of *L. pennellii* and IL8-2-1 genomic DNA hybridized to the cLEC73K23 cDNA are identical, whereas *L. esculentum* M82 restriction patterns are different.

Figures 11A and 11B show a full-length coding sequence (SEQ ID NO. 8) and amino acid sequence (SEQ ID NO. 9) of an *L. pennellii* phenylalanine decarboxylase similar to cLEC73K23 of the present invention.

Figures 12A and 12B show an amino acid sequence alignment of *L. esculentum* cLEC73K23 (SEQ ID NO. 7), *L. esculentum* cLEC75E21 (SEQ ID NO. 5) and the *L. pennellii* cLEC73K23 homolog (SEQ ID NO. 9).

Figures 13A, 13B, and 13C show gas chromatography profiles of volatile compounds extracted from *E. coli* cultures expressing the tomato aromatic amino acid decarboxylase genes cLEC73K23 and cLEC75E21. *E. coli* expressing these tomato genes produce phenethylamine in media containing phenylalanine, while control *E. coli* cultures do not.

Brief Description of the Sequences

SEQ ID NO. 1 shows a nucleotide sequence encoding a 2-phenylethanol dehydrogenase according to the present invention.

SEQ ID NO. 2 shows an amino acid sequence of a 2-phenylethanol dehydrogenase encoded by SEQ ID NO. 1 of the present invention.

SEQ ID NO. 3 shows an oligonucleotide PCR primer that can be used according to the present invention.

SEQ ID NO. 4 shows a nucleotide sequence encoding a phenylalanine decarboxylase according to the present invention.

SEQ ID NO. 5 shows an amino acid sequence of a phenylalanine decarboxylase encoded by SEQ ID NO. 4 of the present invention.

SEQ ID NO. 6 shows a nucleotide sequence encoding a phenylalanine decarboxylase according to the present invention.

SEQ ID NO. 7 shows an amino acid sequence of a phenylalanine decarboxylase encoded by SEQ ID NO. 6 of the present invention.

SEQ ID NO. 8 shows a nucleotide sequence encoding a phenylalanine decarboxylase according to the present invention.

SEQ ID NO. 9 shows an amino acid sequence of a phenylalanine decarboxylase encoded by SEQ ID NO. 8 of the present invention.

Detailed Disclosure of the Invention

The subject invention concerns polynucleotides encoding a plant 2-phenylethanol dehydrogenase enzyme. In one embodiment, the polynucleotide encodes a 2-phenylethanol dehydrogenase of tomato. In an exemplified embodiment, the polynucleotide encodes a tomato 2-phenylethanol dehydrogenase polypeptide having an amino acid sequence shown in SEQ ID NO. 2, or an enzymatically functional fragment or variant thereof. In a specific embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 2 comprises the nucleotide sequence shown in SEQ ID NO. 1, or a sequence encoding an enzymatically functional fragment or variant of SEQ ID NO. 2. In one embodiment, the polynucleotide

encoding the amino acid sequence shown in SEQ ID NO. 2 comprises nucleotides 172 to 1155 of the nucleotide sequence shown in SEQ ID NO. 1.

The subject invention also concerns polynucleotides encoding a plant phenylalanine decarboxylase enzyme. In one embodiment, the polynucleotide encodes a phenylalanine decarboxylase of tomato. In an exemplified embodiment, the polynucleotide encodes a tomato phenylalanine decarboxylase polypeptide having an amino acid sequence shown in SEQ ID NO. 5, 7, or 9, or an enzymatically functional fragment or variant thereof. In a specific embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 5, 7, or 9 comprises the nucleotide sequence shown in SEQ ID NO. 4, 6, or 8, or a sequence encoding an enzymatically functional fragment or variant of SEQ ID NO. 5, 7, or 9. In one embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 5 comprises nucleotides 1 to 1395 of the nucleotide sequence shown in SEQ ID NO. 4. In another embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 7 or 9 comprises nucleotides 1 to 1413 of the nucleotide sequence shown in SEQ ID NO. 6 or 8.

The subject invention also concerns polynucleotide expression constructs comprising a polynucleotide sequence of the present invention encoding a plant 2-phenylethanol dehydrogenase. In one embodiment, an expression construct of the invention comprises a polynucleotide sequence encoding a tomato 2-phenylethanol dehydrogenase comprising an amino acid sequence shown in SEQ ID NO. 2, or an enzymatically functional fragment or variant thereof. In a specific embodiment, the polynucleotide sequence comprises a polynucleotide sequence shown in SEQ ID NO. 1, or a sequence encoding an enzymatically functional fragment or variant of SEQ ID NO. 2. In one embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 2 comprises nucleotides 172 to 1155 of the nucleotide sequence shown in SEQ ID NO. 1.

The subject invention also concerns polynucleotide expression constructs comprising a polynucleotide sequence of the present invention encoding a plant phenylalanine decarboxylase. In one embodiment, an expression construct of the invention comprises a polynucleotide sequence encoding a tomato phenylalanine decarboxylase comprising an amino acid sequence shown in SEQ ID NO. 5, 7, or 9, or an enzymatically functional fragment or variant thereof. In a specific embodiment, the polynucleotide sequence comprises a polynucleotide sequence shown

in SEQ ID NO. 4, 6, or 8, or a sequence encoding an enzymatically functional fragment or variant of SEQ ID NO. 5, 7, or 9. In one embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 5 comprises nucleotides 1 to 1395 of the nucleotide sequence shown in SEQ ID NO. 4. In another embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 7 or 9 comprises nucleotides 1 to 1413 of the nucleotide sequence shown in SEQ ID NO. 6 or 8.

Expression constructs of the invention generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Thus, a person of ordinary skill in the art can select regulatory elements for use in bacterial host cells, yeast host cells, plant host cells, insect host cells, mammalian host cells, and human host cells. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements. As used herein, the term "expression construct" refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. As used herein, the term "operably linked" refers to a juxtaposition of the components described wherein the components are in a relationship that permits them to function in their intended manner. In general, operably linked components are in contiguous relation.

An expression construct of the invention can comprise a promoter sequence operably linked to a polynucleotide sequence encoding a 2-phenylethanol dehydrogenase or phenylalanine decarboxylase of the invention. Promoters can be incorporated into a polynucleotide using standard techniques known in the art. Multiple copies of promoters or multiple promoters can be used in an expression construct of the invention. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site in the expression construct as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

If the expression construct is to be provided in or introduced into a plant cell, then plant viral promoters, such as, for example, a cauliflower mosaic virus (CaMV) 35S (including the enhanced CaMV 35S promoter (see, for example U.S. Patent No. 5,106,739)) or a CaMV 19S promoter can be used. Other promoters that can be used for expression constructs in plants

include, for example, prolifera promoter, Ap3 promoter, heat shock promoters, T-DNA 1'- or 2'-promoter of *A. tumefaciens*, polygalacturonase promoter, chalcone synthase A (CHS-A) promoter from petunia, tobacco PR-1a promoter, ubiquitin promoter, actin promoter, alcA gene promoter, pin2 promoter (Xu *et al.*, 1993), maize WipI promoter, maize trpA gene promoter (U.S. Patent No. 5,625,136), maize CDPK gene promoter, and RUBISCO SSU promoter (U.S. Patent No. 5,034,322) can also be used. Tissue-specific promoters, for example fruit-specific promoters, such as the E8 promoter of tomato (accession number: AF515784; Good *et al.* (1994)) and the TPRP-F1 promotoer (Salts *et al.*, 1991; Santino *et al.*, 1997) can be used. U.S. Patent No. 5,753,475 describes fruit-specific promoter sequences. Flower organ-specific promoters can be used with an expression construct of the present invention for expressing a polynucleotide of the invention in the flower organ of a plant. Examples of flower organ-specific promoters include any of the promoter sequences described in U.S. Patent Nos. 6,462,185; 5,639,948; and 5,589,610. Seed-specific promoters such as the promoter from a β-phaseolin gene (of kidney bean) or a glycinin gene (of soybean), and others, can also be used. Constitutive promoters (such as the CaMV, ubiquitin, actin, or NOS promoter), developmentally-regulated promoters, and inducible promoters (such as those promoters than can be induced by heat, light, hormones, or chemicals) are also contemplated for use with polynucleotide expression constructs of the invention.

For expression in animal cells, an expression construct of the invention can comprise suitable promoters that can drive transcription of the polynucleotide sequence. If the cells are mammalian cells, then promoters such as, for example, actin promoter, metallothionein promoter, NF-kappaB promoter, EGR promoter, SRE promoter, IL-2 promoter, NFAT promoter, osteocalcin promoter, SV40 early promoter and SV40 late promoter, Lck promoter, BMP5 promoter, TRP-1 promoter, murine mammary tumor virus long terminal repeat promoter, STAT promoter, or an immunoglobulin promoter can be used in the expression construct. The baculovirus polyhedrin promoter can be used with an expression construct of the invention for expression in insect cells.

For expression in prokaryotic systems, an expression construct of the invention can comprise promoters such as, for example, alkaline phosphatase promoter, tryptophan (trp)

promoter, lambda P_L promoter, β-lactamase promoter, lactose promoter, phoA promoter, T3 promoter, T7 promoter, or tac promoter (de Boer *et al.*, 1983).

Promoters suitable for use with an expression construct of the invention in yeast cells include, but are not limited to, 3-phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase promoter, metallothionein promoter, alcohol dehydrogenase-2 promoter, and hexokinase promoter.

Expression constructs of the invention may optionally contain a transcription termination sequence, a translation termination sequence, a sequence encoding a signal peptide, and/or enhancer elements. Transcription termination regions can typically be obtained from the 3' untranslated region of a eukaryotic or viral gene sequence. Transcription termination sequences can be positioned downstream of a coding sequence to provide for efficient termination. A signal peptide sequence is a short amino acid sequence typically present at the amino terminus of a protein that is responsible for the relocation of an operably linked mature polypeptide to a wide range of post-translational cellular destinations, ranging from a specific organelle compartment to sites of protein action and the extracellular environment. Targeting gene products to an intended cellular and/or extracellular destination through the use of an operably linked signal peptide sequence is contemplated for use with the polypeptides of the invention. Classical enhancers are cis-acting elements that increase gene transcription and can also be included in the expression construct. Classical enhancer elements are known in the art, and include, but are not limited to, the CaMV 35S enhancer element, cytomegalovirus (CMV) early promoter enhancer element, and the SV40 enhancer element. Intron-mediated enhancer elements that enhance gene expression are also known in the art. These elements must be present within the transcribed region and are orientation dependent. Examples include the maize shrunken-1 enhancer element (Clancy and Hannah, 2002).

DNA sequences which direct polyadenylation of mRNA transcribed from the expression construct can also be included in the expression construct, and include, but are not limited to, an octopine synthase or nopaline synthase signal. The expression constructs of the invention can also include a polynucleotide sequence that directs transposition of other genes, *i.e.*, a transposon.

Expression constructs can also include one or more dominant selectable marker genes, including, for example, genes encoding antibiotic resistance and/or herbicide-resistance for selecting transformed cells. Antibiotic-resistance genes can provide for resistance to one or more of the following antibiotics: hygromycin, kanamycin, bleomycin, G418, streptomycin, paromomycin, neomycin, and spectinomycin. Kanamycin resistance can be provided by neomycin phosphotransferase (NPT II). Herbicide-resistance genes can provide for resistance to phosphinothricin acetyltransferase or glyphosate. Other markers used for cell transformation screening include genes encoding β-glucuronidase (GUS), β-galactosidase, luciferase, nopaline synthase, chloramphenicol acetyltransferase (CAT), green fluorescence protein (GFP), or enhanced GFP (Yang *et al.*, 1996).

The subject invention also concerns polynucleotide vectors comprising a polynucleotide sequence of the invention that encodes a 2-phenylethanol dehydrogenase or phenylalanine decarboxylase. Unique restriction enzyme sites can be included at the 5' and 3' ends of an expression construct or polynucleotide of the invention to allow for insertion into a polynucleotide vector. As used herein, the term "vector" refers to any genetic element, including for example, plasmids, cosmids, chromosomes, phage, virus, and the like, which is capable of replication when associated with proper control elements and which can transfer polynucleotide sequences between cells. Vectors contain a nucleotide sequence that permits the vector to replicate in a selected host cell. A number of vectors are available for expression and/or cloning, and include, but are not limited to, pBR322, pUC series, M13 series, and pBLUESCRIPT vectors (Stratagene, La Jolla, CA).

Polynucleotides of the present invention can be composed of either RNA or DNA. Preferably, the polynucleotides are composed of DNA. The subject invention also encompasses those polynucleotides that are complementary in sequence to the polynucleotides disclosed herein.

Because of the degeneracy of the genetic code, a variety of different polynucleotide sequences can encode 2-phenylethanol dehydrogenase enzymes of the present invention. A table showing all possible triplet codons (and where U also stands for T) and the amino acid encoded by each codon is described in Lewin (1985). In addition, it is well within the skill of a person trained in the art to create alternative polynucleotide sequences encoding the same, or essentially

the same, 2-phenylethanol dehydrogenase or phenylalanine decarboxylase enzymes of the subject invention. These variant or alternative polynucleotide sequences are within the scope of the subject invention. As used herein, references to "essentially the same" sequence refers to sequences which encode amino acid substitutions, deletions, additions, or insertions which do not materially alter the functional activity of the polypeptide encoded by the polynucleotides of the present invention. Allelic variants of the nucleotide sequences encoding a 2-phenylethanol dehydrogenase or phenylalanine decarboxylase of the invention are also encompassed within the scope of the invention.

The subject invention also concerns an isolated plant 2-phenylethanol dehydrogenase. In one embodiment, the 2-phenylethanol dehydrogenase is a 2-phenylethanol dehydrogenase of tomato. In a specific embodiment, the 2-phenylethanol dehydrogenase has an amino acid sequence as shown in SEQ ID NO. 2, or an enzymatically functional fragment or variant thereof. A 2-phenylethanol dehydrogenase enzyme of the invention can be purified using standard techniques known in the art. In one embodiment, a polynucleotide of the invention encoding a 2-phenylethanol dehydrogenase is incorporated into a microorganism such as *E. coli* and the 2-phenylethanol dehydrogenase expressed and then isolated therefrom.

The subject invention also concerns an isolated plant phenylalanine decarboxylase. In one embodiment, the phenylalanine decarboxylase is a phenylalanine decarboxylase of tomato. In a specific embodiment, the phenylalanine decarboxylase has an amino acid sequence as shown in SEQ ID NO. 5, 7, or 9, or an enzymatically functional fragment or variant thereof. A phenylalanine decarboxylase enzyme of the invention can be purified using standard techniques known in the art. In one embodiment, a polynucleotide of the invention encoding a phenylalanine decarboxylase is incorporated into a microorganism such as *E. coli* and the phenylalanine decarboxylase expressed and then isolated therefrom.

Polypeptide fragments according to the subject invention typically comprise a contiguous span of about or at least 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134,

135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, or 327 amino acids of SEQ ID NO. 2; or a contiguous span of about or at least 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406,

407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, or 464 amino acids of SEQ ID NO. 5; or a contiguous span of about or at least 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, or 470 amino acids of SEQ ID NO. 7 or 9.

Polypeptide fragments of the subject invention can be any integer in length from at least about 25 consecutive amino acids to 1 amino acid less than the sequence shown in SEQ ID NO. 2, 5, 7, or 9. Thus, for SEQ ID NO. 2, a polypeptide fragment can be any integer of consecutive

amino acids from about 25 to 327 amino acids; for SEQ ID NO. 5, a polypeptide fragment can be any integer of consecutive amino acids from about 25 to 464 amino acids; for SEQ ID NO. 7 or 9, a polypeptide fragment can be any integer of consecutive amino acids from about 25 to 470 amino acids. The term "integer" is used herein in its mathematical sense and thus representative integers include: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, and/or 470.

Each polypeptide fragment of the subject invention can also be described in terms of its N-terminal and C-terminal positions. For example, combinations of N-terminal to C-terminal fragments of about 25 contiguous amino acids to 1 amino acid less than the full length

polypeptide of SEQ ID NO. 2, 5, 7, and 9 are included in the present invention. Thus, using SEQ ID NO. 2 as an example, a 25 consecutive amino acid fragment could correspond to amino acids of SEQ ID NO. 2 selected from the group consisting of 1-25, 2-26, 3-27, 4-28, 5-29, 6-30, 7-31, 8-32, 9-33, 10-34, 11-35, 12-36, 13-37, 14-38, 15-39, 16-40, 17-41, 18-42, 19-43, 20-44, 21-45, 22-46, 23-47, 24-48, 25-49, 26-50, 27-51, 28-52, 29-53, 30-54, 31-55, 32-56, 33-57, 34-58, 35-59, 36-60, 37-61, 38-62, 39-63, 40-64, 41-65, 42-66, 43-67, 44-68, 45-69, 46-70, 47-71, 48-72, 49-73, 50-74, 51-75, 52-76, 53-77, 54-78, 55-79, 56-80, 57-81, 58-82, 59-83, 60-84, 61-85, 62-86, 63-87, 64-88, 65-89, 66-90, 67-91, 68-92, 69-93, 70-94, 71-95, 72-96, 73-97, 74-98, 75-99, 76-100, 77-101, 78-102, 79-103, 80-104, 81-105, 82-106, 83-107, 84-108, 85-109, 86-110, 87-111, 88,-112, 89-113, 90-114, 91-115, 92-116, 93-117, 94-118, 95-119, 96-120, 97-121, 98-122, 99-123, 100-124, 101-125, 102-126, 103-127, 104-128, 105-129, 106-130, 107-131, 108-132, 109-133, 110-134, 111-135, 112-136, 113-137, 114-138, 115-139, 116-140, 117-141, 118-142, 119-143, 120-144, 121-145, 122-146, 123-147, 124-148, 125-149, 126-150, 127-151, 128-152, 129-153, 130-154, 131-155, 132-156, 133-157, 134-158, 135-159, 136-160, 137-161, 138-162, 139-163, 140-164, 141-165, 142-166, 143-167, 144-168, 145-169, 146-170, 147-171, 148-172, 149-173, 150-174, 151-175, 152-176, 153-177, 154-178, 155-179, 156-180, 157-181, 158-182, 159-183, 160-184, 161-185, 162-186, 163-187, 164-188, 165-189, 166-190, 167-191, 168-192, 169-193, 170-194, 171-195, 172-196, 173-197, 174-198, 175-199, 176-200, 177-201, 178-202, 179-203, 180-204, 181-205, 182-206, 183-207, 184-208, 185-209, 186-210, 187-211, 188-212, 189-213, 190-214, 191-215, 192-216, 193-217, 194-218, 195-219, 196-220, 197-221, 198-222, 199-223, 200-224, 201-225, 202-226, 203-227, 204-228, 205-229, 206-230, 207-231, 208-232, 209-233, 210-234, 211-235, 212-236, 213-237, 214-238, 215-239, 216-240, 217-241, 218-242, 219-243, 220-244, 221-245, 222-246, 223-247, 224-248, 225-249, 226-250, 227-251, 228-252, 229-253, 230-254, 231-255, 232-256, 233-257, 234-258, 235-259, 236-260, 237-261, 238-262, 239-263, 240-264, 241-265, 242-266, 243-267, 244-268, 245-269, 246-270, 247-271, 248-272, 249-273, 250-274, 251-275, 252-276, 253-277, 254-278, 255-279, 256-280, 257-281, 258-282, 259-283, 260-284, 261-285, 262-286, 263-287, 264-288, 265-289, 266-290, 267-291, 268-292, 269-293, 270-294, 271-295, 272-296, 273-297, 274-298, 275-299, 276-300, 277-301, 278-302, 279-303, 280-304, 281-305, 282-306, 283-307, 284-308, 285-309, 286-310, 287-311, 288-312, 289-313, 290-314, 291-315, 292-316, 293-317, 294-318, 295-319, 296-320, 297-321,

298-322, 299-323, 300-324, 301-325, 302-326, 303-327, and 304-328. Similarly, the amino acids corresponding to all other fragments of sizes between 26 consecutive amino acids and 327 consecutive amino acids of SEQ ID NO. 2 are included in the present invention and can also be immediately envisaged based on these examples. Therefore, additional examples, illustrating various fragments of the polypeptides of SEQ ID NO. 2 are not individually listed herein in order to avoid unnecessarily lengthening the specification. Fragment embodiments as decribed above are also contemplated for the polypeptides of SEQ ID NO. 5, 7, and 9, taking into account that the polypeptides are 465, 471, and 471 amino acids in length, respectively, and are not individually listed herein in order to avoid unnecessarily lengthening the specification.

Polypeptide fragments comprising:

a) 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, or 327 consecutive amino acids of SEQ ID NO. 2;

b) 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117,

118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, and 464 consecutive amino acids of SEQ ID NO. 5; and

c) 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250,

251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, and 470 consecutive amino acids of SEQ ID NO. 7 or 9 may alternatively be described by the formula "n to c" (inclusive), where "n" equals the N-terminal amino acid position and "c" equals the C-terminal amino acid position of the polypeptide. In this embodiment of the invention, "n" is an integer having a lower limit of 1 and an upper limit of the total number of amino acids of the full length polypeptide minus 24 (*e.g.*, 328-24=304 for SEQ ID NO. 2). "c" is an integer between 25 and the number of amino acids of the full length polypeptide sequence (328 for SEQ ID NO. 2) and "n" is an integer smaller then "c" by at least 24. Therefore, for SEQ ID NO. 2, "n" is any integer selected from the list consisting of: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235,

236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, and 304; and "c" is any integer selected from the group consisting of: 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, and 328 provided that "n" is a value less than "c" by at least 24. Every combination of "n" and "c" positions are included as specific embodiments of polypeptide fragments of the invention. All ranges used to describe any polypeptide fragment embodiment of the present invention are inclusive unless specifically set forth otherwise.

Fragments of a plant 2-phenylethanol dehydrogenase or phenylalanine decarboxylase of the invention, as described herein, can be obtained by cleaving the polypeptides of the invention with a proteolytic enzyme (such as trypsin, chymotrypsin, or collagenase) or with a chemical reagent, such as cyanogen bromide (CNBr). Alternatively, polypeptide fragments can be generated in a highly acidic environment, for example at pH 2.5. Polypeptide fragments can also be prepared by chemical synthesis or using host cells transformed with an expression vector comprising a polynucleotide encoding a fragment of a 2-phenylethanol dehydrogenase or

phenylalanine decarboxylase enzyme of the invention, for example, a 2-phenylethanol dehydrogenase that is a fragment of the amino acid sequence shown in SEQ ID NO. 2, or a phenylalanine decarboxylase that is a fragment of the amino acid sequence shown in SEQ ID NO. 5, 7, or 9.

Substitution of amino acids other than those specifically exemplified or naturally present in a plant 2-phenylethanol dehydrogenase or phenylalanine decarboxylase enzyme of the invention are also contemplated within the scope of the present invention. For example, non-natural amino acids can be substituted for the amino acids of 2-phenylethanol dehydrogenase or phenylalanine decarboxylase, so long as the 2-phenylethanol dehydrogenase or phenylalanine decarboxylase enzyme having the substituted amino acids retains substantially the same biological activity as the 2-phenylethanol dehydrogenase or phenylalanine decarboxylase in which amino acids have not been substituted. Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ-amino butyric acid, ε-amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, τ-butylglycine, τ-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form. Allelic variants of a protein sequence of 2-phenylethanol dehydrogenase and phenylalanine decarboxylase enzymes of the present invention are also encompassed within the scope of the invention.

Amino acids can be generally categorized in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an 2-phenylethanol dehydrogenase or phenylalanine decarboxylase enzyme of the present invention having an amino acid of one class is replaced with another amino acid of the same class fall within the scope of the subject invention so long as the 2-phenylethanol dehydrogenase or phenylalanine decarboxylase enzyme having the substitution still retains substantially the same biological activity as the 2-phenylethanol dehydrogenase or phenylalanine decarboxylase enzyme that does not have the

substitution. Polynucleotides encoding a 2-phenylethanol dehydrogenase or phenylalanine decarboxylase enzyme having one or more amino acid substitutions in the sequence are contemplated within the scope of the present invention. Table 3 below provides a listing of examples of amino acids belonging to each class.

Table 3.

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

The subject invention also concerns variants of the polynucleotides of the present invention that encode enzymatically active 2-phenylethanol dehydrogenase or phenylalanine decarboxylase enzymes of the invention. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted. The nucleotides that can be substituted for natural nucleotides of DNA have a base moiety that can include, but is not limited to, inosine, 5-fluorouracil, 5-bromouracil, hypoxanthine, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified and includes, but is not limited to, arabinose, xylulose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

Fragments and variants of 2-phenylethanol dehydrogenase and phenylalanine decarboxylase of the present invention can be generated as described herein and tested for the presence of enzymatic function using standard techniques known in the art. For example, for testing fragments and/or variants of a 2-phenylethanol dehydrogenase, the conversion of

phenylacetaldehyde to 2-phenylethanol can be assayed according to the present invention. Thus, an ordinarily skilled artisan can readily prepare and test fragments and variants of a 2-phenylethanol dehydrogenase of the invention and determine whether the fragment or variant retains functional enzymatic activity relative to full-length or a wildtype plant 2-phenylethanol dehydrogenase. Similarly, an assay for the conversion of phenylalanine to phenethylamine can be used to assess enzymatic activity of fragments and/or variants of phenylalanine decarboxylase of the present invention.

Polynucleotides and polypeptides contemplated within the scope of the subject invention can also be defined in terms of more particular identity and/or similarity ranges with those sequences of the invention specifically exemplified herein. The sequence identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. *See* NCBI/NIH website.

The subject invention also contemplates those polynucleotide molecules having sequences which are sufficiently homologous with the polynucleotide sequences exemplified herein so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis *et al.*, 1982). As used herein, "stringent" conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25 C below the melting temperature (Tm) of the DNA hybrid in 6x SSPE, 5x Denhardt's

solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature, Tm, is described by the following formula (Beltz *et al.*, 1983):

Tm=81.5 C+16.6 Log[Na+]+0.41(%G+C)-0.61(% formamide)-600/length of duplex in base pairs.

Washes are typically carried out as follows:

(1) Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).

(2) Once at Tm-20 C for 15 minutes in 0.2x SSPE, 0.1% SDS (moderate stringency wash).

As used herein, the terms "nucleic acid" and "polynucleotide" refer to a deoxyribonucleotide, ribonucleotide, or a mixed deoxyribonucleotide and ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally-occurring nucleotides. The polynucleotide sequences include the DNA strand sequence that is transcribed into RNA and the strand sequence that is complementary to the DNA strand that is transcribed. The polynucleotide sequences also include both full-length sequences as well as shorter sequences derived from the full-length sequences. Allelic variations of the exemplified sequences also fall within the scope of the subject invention. The polynucleotide sequence includes both the sense and antisense strands either as individual strands or in the duplex.

The subject invention also concerns cells transformed with a polynucleotide of the present invention encoding a 2-phenylethanol dehydrogenase of the invention. In one embodiment, the cell is transformed with a polynucleotide sequence comprising a sequence encoding the amino acid sequence shown in SEQ ID NO. 2, or an enzymatically functional fragment or variant thereof. In a specific embodiment, the cell is transformed with a polynucleotide sequence shown in SEQ ID NO. 1, or a sequence encoding an enzymatically functional fragment or variant of SEQ ID NO. 2. In one embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 2 comprises nucleotides 172 to 1155 of the nucleotide sequence shown in SEQ ID NO. 1.

The subject invention also concerns cells transformed with a polynucleotide of the present invention encoding a phenylalanine decarboxylase of the invention. In one embodiment, the cell is transformed with a polynucleotide sequence comprising a sequence encoding the amino acid sequence shown in SEQ ID NO. 5, 7, or 9, or an enzymatically functional fragment or variant thereof. In a specific embodiment, the cell is transformed with a polynucleotide sequence shown in SEQ ID NO. 4, 6, or 8, or a sequence encoding an enzymatically functional fragment or variant of SEQ ID NO. 5, 7, or 9. In one embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 5 comprises nucleotides 1 to 1395 of the nucleotide sequence shown in SEQ ID NO. 4. In another embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 7 or 9 comprises nucleotides 1 to 1413 of the nucleotide sequence shown in SEQ ID NO. 6 or 8.

Preferably, the polynucleotide sequence is provided in an expression construct of the invention. The transformed cell can be a prokaryotic cell, for example, a bacterial cell such as *E. coli* or *B. subtilis*, or the transformed cell can be a eukaryotic cell, for example, a plant cell, including protoplasts, or an animal cell. Plant cells include, but are not limited to, dicotyledonous, monocotyledonous, and conifer cells. In one embodiment, the plant cell is a cell from tomato. Animal cells include human cells, mammalian cells, avian cells, and insect cells. Mammalian cells include, but are not limited to, COS, 3T3, and CHO cells.

Plants, plant tissues, and plant cells transformed with or bred to contain a polynucleotide of the invention are also contemplated by the present invention. Plants within the scope of the present invention include monocotyledonous plants, such as, for example, rice, wheat, barley, oats, sorghum, maize, sugarcane, pineapple, onion, bananas, coconut, lilies, grasses, and millet. Plants within the scope of the present invention also include dicotyledonous plants, such as, for example, tomato, peas, alfalfa, melon, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple trees, grape, cotton, sunflower, and lettuce; and conifers. Preferably, the plant, plant tissue, or plant cell is tomato. Ornamental and herb plants containing a polynucleotide of the invention are also contemplated within the scope of the invention. Ornamental plants include roses, petunia, carnations, orchids, tulips, and the like. Herb plants include parsley, sage, rosemary, thyme, and the like. Techniques for transforming plant cells with a gene are known in the art and include, for example, *Agrobacterium* infection,

biolistic methods, electroporation, calcium chloride treatment, *etc.* Transformed cells can be selected, redifferentiated, and grown into plants using standard methods known in the art. The seeds and progeny of any transformed or transgenic plant cells or plants of the invention are also included within the scope of the present invention.

The subject invention also concerns methods for providing a plant with increased flavor or fragrance of fruit or flower by incorporating one or more polynucleotide of the present invention in the genome of the plant cells and expressing the polypeptide encoded by the polynucleotide. In one embodiment, a plant is grown from a transformed plant cell of the invention. Preferably, the polynucleotide encodes a 2-phenylethanol dehydrogenase or a phenylalanine decarboxylase derived from the same plant species as the plant. In one embodiment, the plant is tomato. In another embodiment, the plant is a rose or other scented ornamental. In those embodiments, where increased flavor of fruit is desired, preferably the polynucleotide(s) of the invention is expressed in the fruit. In those embodiments where increased or enhanced fragrance of fruit or flower is desired, preferably the polynucleotide(s) of the invention is expressed in the fruit and/or flower. In a specific embodiment, a polynucleotide encoding an amino acid sequence shown in SEQ ID NO. 2, or an enzymatically functional fragment or variant thereof, is incorporated into a tomato plant genome. In a specific embodiment, the polynucleotide comprises a nucleotide sequence shown in SEQ ID NO. 1, or a sequence encoding an enzymatically functional fragment or variant of SEQ ID NO. 2. In one embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 2 comprises nucleotides 172 to 1155 of the nucleotide sequence shown in SEQ ID NO. 1. In another specific embodiment, a polynucleotide encoding an amino acid sequence shown in SEQ ID NO. 5, 7, or 9, or an enzymatically functional fragment or variant thereof, is incorporated into a tomato plant genome. In a specific embodiment, the polynucleotide comprises a nucleotide sequence shown in SEQ ID NO. 4, 6, or 8, or a sequence encoding an enzymatically functional fragment or variant of SEQ ID NO. 5, 7, or 9. In one embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 5 comprises nucleotides 1 to 1395 of the nucleotide sequence shown in SEQ ID NO. 4. In another embodiment, the polynucleotide encoding the amino acid sequence shown in SEQ ID NO. 7 or 9 comprises nucleotides 1 to 1413 of the nucleotide sequence shown in SEQ ID NO. 6 or 8. The level of expression of a

polynucleotide of the invention can be manipulated using standard methods known in the art, including the use of promoters that provide for low, intermediate or high levels of expression.

The subject invention also concerns methods for producing 2-phenylethanol. In one embodiment, recombinantly produced 2-phenylethanol dehydrogenase of the invention can be used to enzymatically convert a suitable substrate, such as phenylacetaldehyde, into 2-phenylethanol. In another embodiment, a microorganism, such as yeast or *E. coli*, can be transformed with and express a polynucleotide encoding a plant 2-phenylethanol dehydrogenase enzyme of the invention and, optionally, one or more enzymes, such as phenylalanine decarboxylase and phenylethylamine oxidase, that through their enzymatic reactions result in a suitable substrate (*e.g.*, phenylacetaldehyde) for 2-phenylethanol dehydrogenase to convert to 2-phenylethanol. Transformed microorganisms can be grown and polynucleotides expressed constitutively or induced, and 2-phenylethanol isolated from the microorganisms.

The subject invention also concerns methods for producing phenethylamine. In one embodiment, recombinantly produced phenylalanine decarboxylase of the invention can be used to enzymatically convert a suitable substrate, such as phenylalanine, into phenethylamine. In another embodiment, a microorganism, such as yeast or *E. coli*, can be transformed with and express a polynucleotide encoding a plant phenylalanine decarboxylase of the invention and, optionally, one or more enzymes, that through their enzymatic reactions result in a suitable substrate (*e.g.*, phenylalanine) for phenylalanine decarboxylase to convert to phenethylamine. Transformed microorganisms can be grown and polynucleotides expressed constitutively or induced, and phenethylamine isolated from the microorganisms.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Materials and Methods

Plant material.

Tomato (*Lycopersicon esculentum* Mill. cv. M82) and *Lycopersicon pennellii* introgression lines 8-2 and 8-2-1 (Eshed and Zamir, 1994) were grown in the greenhouse or field

under standard conditions. Petunia plants were grown in a greenhouse under standard conditions.

Volatile collection.

Volatiles were collected from approximately 100g of chopped ripe tomato fruit as described by Schmelz *et al.* (2003). Petunia volatiles were collected from five flowers from each plant harvested at dusk. Volatiles were separated on an Agilent DB-5 column and analyzed on an Agilent 6890N gas chromatograph.

Microarrays.

Tomato cDNA microarrays were as described in Moore *et al.* (2002). Total RNAs were isolated as described earlier (Ciardi *et al.*, 2000). Arrays were hybridized with Cy3 or Cy5 labeled cDNAs from M82 and introgression line 8-2-1 fruit. Arrays were performed multiple times and with dyes reversed to ensure accuracy of the expression data.

2-phenylethanol dehydrogenase expression in *E. coli*.

A full-length 2-phenylethanol dehydrogenase cDNA was cloned by 5' RACE from tomato fruit cDNA using primer 5'-TCCTTGGCCCCACCAAGAGAAAGCAAGTGCTGCGT-3' (SEQ ID NO. 3). Following sequence analysis the full-length cDNA was obtained by PCR. The coding region was cloned into vector pDEST15 containing a glutathione S-transferase (GST) tag (Invitrogen) by recombination, and transformed into *E. coli* strain BL21-SI (Invitrogen) for inducible protein expression. Enzyme activity of crude *E. coli* extracts was determined by the method of Larroy *et al.* (2002) using phenylacetaldehyde, cinnamaldehyde or vanillin as a substrate.

Production of transgenic petunia plants.

The full-length 2-phenylethanol dehydrogenase cDNA (SEQ ID NO. 1) was cloned in a vector under the control of the figwort mosaic virus 35S promoter (Richins *et al.*, 1987) and followed by the Agrobacterium nopaline synthase (*nos*) 3' terminator. The transgene was

introduced into *Petunia hybrida* cv. Mitchell Diploid by the method of Wilkinson *et al.* (1997) with kanamycin resistance as a selectable marker.

In vivo phenylalanine decarboxylase activity assays.

Tomato (M82 or introgression line 8-2-1) fruit pericarp disks were incubated with 1μCi universally-labeled ^{14}C-phenylalanine for 8hr. Production of ^{14}C-CO_2 was measured by incubating the pericarp disk in a sealed flask with a 2N KOH filter paper disk suspended above the pericarp disk, followed by scintillation counting. ^{14}C-phenylalanine and ^{14}C-phenylethylamine were extracted from the pericarp disk and separated using an AG-1 (OH-) column in series with a BioRex-70 column as described by Rontein *et al.* (2001). Production of ^{14}C-phenylethylamine was confirmed by thin layer chromatography.

Phenylalanine decarboxylase expression in *E. coli*.

The full-length aromatic amino acid decarboxylase cDNAs were cloned by sequencing putative clones from the TIGR database. Following sequence analysis the full-length coding sequence was obtained by PCR, and cloned into vector pENTR/D-TOPO. The coding region was then cloned into vector pDEST15 containing a GST tag (Invitrogen) by recombination, and transformed into *E. coli* strain BL21-AI (Invitrogen) for inducible protein expression. Production of the recombinant protein was confirmed by protein blotting with anti-GST antibodies. Enzyme activity was determined by growing *E. coli* strains expressing the aromatic amino acid decarboxylases in media containing 19.4mM phenylalanine. Volatile compounds were extracted from the cultures using an equal volume of hexanes. Extracts were concentrated and analyzed by gas chromatography on an Agilent DB-5 column on an Agilent 6890N gas chromatograph. Identification of phenethylamine was confirmed by GC-mass spectrometry as described by Schmelz *et al.* (2001).

Southern blotting.

DNA from *L. esculentum* M82 or 8-2-1 or *L. pennellii* leaves was digested with *EcoRI*, *EcoRV*, *DraI*, *HaeIII* or *ScaI*. Southern blotting was performed as described by Sambrook *et al.* (1989).

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1—Volatiles Analysis

Volatiles analysis by GC on a DB5 column indicated high levels of phenylacetaldehyde and 2-phenylethanol in ripe fruit from L. pennellii introgression line (IL) 8-2-1. Levels of other tomato volatiles were similar to control M82 ripe fruit (Figure 1). Levels of 2-phenylethanol were approximately 250X higher in 8-2-1 fruit than in M82 fruit. Phenylacetaldehyde levels in IL8-2-1 fruit were approximately 20X higher than in control fruit (Figure 2). IL8-2-1 fruit had a distinct floral (rose-like) aroma consistent with the floral aromas of phenylacetaldehyde and 2-phenylethanol.

Example 2—Microarray Analysis and Isolation of 2-Phenylethanol Dehydrogenase

Microarrays containing approximately 3,000 tomato cDNAs indicated that an alcohol dehydrogenase gene (clone cLET2M9) was more highly expressed in IL8-2-1 fruit than in control M82 fruit; whereas, a related tomato alcohol dehydrogenase gene was not upregulated in IL8-2-1 fruit (Table 4).

Table 4. Microarray gene expression data for two alcohol dehydrogenase–like genes

Microarray clone	Ratio
cLET2M9	+2.2
cLEG71F12	-2.35

RNAs extracted from M82 and introgression line 8-2-1 fruit were compared using cDNA microarrays. Positive values indicate higher RNA expression levels in introgression line 8-2-1 fruit; negative values indicate higher expression in M82 fruit.

Since clone cLET2M9 was only a partial cDNA, the full-length cDNA sequence for this clone was obtained by 5' RACE. The full-length cDNA sequence of 2-phenylethanol dehydrogenase was then obtained by PCR, and confirmed by sequence analysis (Figure 3A) (SEQ ID NO. 1). The deduced amino acid sequence of the 2-phenylethanol dehydrogenase is

shown in Figure 3B (SEQ ID NO. 2). In plants, a substrate such as phenylacetaldehyde can be converted to 2-phenylethanol by 2-phenylethanol dehydrogenase (Figure 4).

Example 3—Enzyme Activity

The 2-phenylethanol dehydrogenase coding region of the full-length cLET2M9 and the related cLEG71F12 were cloned in vector pDEST15 with a GST tag and transformed into *E. coli* BL21-SI cells for inducible expression. The production of recombinant protein in *E. coli* was determined by Western blotting with an anti-GST antibody. Alcohol dehydrogenase activities on phenylacetaldehyde and several related substrates were determined spectrophotometrically by the reduction in levels of NADPH and a decrease in OD_{340} (Figure 5). The highest level of activity was observed with phenylacetaldehyde as a substrate. Lower activity levels were also observed with cinnamaldehyde as a substrate, whereas negligible activity was seen with vanillin as a substrate. Protein from cLEG71F12 expressed in *E. coli* showed very little activity on the three substrates tested.

Example 4—Expression of 2-Phenylethanol Dehydrogenase in Transgenic Petunia

Full-length tomato 2-phenylethanol dehydrogenase cDNA was introduced into petunia (cv. Mitchell Diploid) under control of the constitutively expressed figwort mosaic virus promoter. Several transgenic petunia lines had high levels of expression of the tomato gene in flowers (data not shown). Wild-type petunia flowers emit relatively high levels of phenylacetaldehyde and lower levels of 2-phenylethanol. However, the transgenic petunia flowers expressing the polynucleotide encoding tomato 2-phenylethanol dehydrogenase have higher levels of 2-phenylethanol and lower levels of phenylacetaldehyde than wild-type flowers (Figure 6). Levels of other petunia flower volatiles were similar to wild-type in the transgenic flowers. A range of phenylacetaldehyde and 2-phenylethanol levels were seen in the transgenic lines, however the majority of the lines had higher levels of 2-phenylethanol and lower levels of phenylacetaldehyde than wild-type flowers (Figure 7). Overall, these data indicate that the introduction of the 2-phenylethanol dehydrogenase tomato transgene results in the conversion of phenylacetaldehyde to 2-phenylethanol in petunia flowers.

Example 5—*In vivo* Aromatic Amino Acid Decarboxylase Activity

A pathway for the formation of 2-phenylethanol in plants is shown in Figure 4. In the pathway phenylalanine is converted to phenethylamine by phenylalanine decarboxylase, followed by conversion of phenethylamine to phenylacetaldehyde by an amine oxidase. Phenylacetaldehyde is then converted to 2-phenylethanol by a 2-phenylethanol dehydrogenase. To establish the first step in this pathway, tomato fruit pericarp disks were incubated with ^{14}C-phenylalanine. The formation of ^{14}C-phenethylamine and ^{14}C-CO_2, the products of the decarboxylase reaction were then determined (Table 5). Higher levels of both phenethylamine and CO_2 were formed in the IL8-2-1 pericarp disks than in the control M82 pericarp disks (Table 5), and correlate with the higher levels of phenylacetaldehyde and 2-phenylethanol in IL8-2-1 tomato fruit. These results indicate that phenethylamine can be an intermediate in the pathway to phenylacetaldehdye and 2-phenylethanol.

Table 5. Phenylalanine decarboxylase activity of M82 and IL8-2-1 tomato pericarp disks fed ^{14}C phenylalanine.

Line	nCi CO_2	nCi phenethylamine
M82	0.96 ± 0.05	0.36 ± 0.02
8-2-1	5.16 ± 2.36	2.08 ± 0.39

Pericarp disks were fed 1 μCi universally labeled ^{14}C-phenylalanine for 8 hours, and amounts of ^{14}C-CO_2 and ^{14}C-phenethylamine produced were determined.

Example 6—Identification of Aromatic Amino Acid Decarboxylase Genes

Conversion of phenylalanine to phenethylamine would be catalyzed by a phenylalanine decarboxylase. Therefore, tomato sequence databases were searched for cDNAs with similarity to other aromatic amino acid decarboxylases. Several clones of *L. esculentum* cDNA sequences were identified, although two were more similar to histidine decarboxylases than aromatic amino acid decarboxylases. The full-length coding sequence of each of these genes was obtained by PCR, and confirmed by sequence analysis.

Example 7—Enzyme Activity

The coding regions of the full-length aromatic amino acid decarboxylases were cloned in vector pDEST15 with a GST tag and transformed into *E.coli* BL21-SI cells for inducible expression. The production of recombinant proteins in *E. coli* was confirmed by Western blotting with an anti-GST antibody. *E. coli* cultures expressing the putative aromatic amino acid decarboxylases were grown in the presence of phenylalanine. Volatile compounds were extracted from these cultures and analyzed by gas chromatography. *E. coli* cultures expressing two putative aromatic amino acid decarboxylases (cLEC73K23 and cLEC75E21) produced a compound with the same retention time as phenethylamine, while control cultures did not (Figure 13). *E. coli* cultures expressing three other putative amino acid decarboxylases did not produce phenethylamine. The presence of phenethylamine in the samples was confirmed by GC-MS. The nucleic acid coding sequences and amino acid sequences of the phenylalanine decarboxylases are shown in Figures 8A-8B (SEQ ID NO. 4 and SEQ ID NO. 5) and Figures 9A-9B (SEQ ID NO. 6 and SEQ ID NO. 7). The cLEC73K23 and cLEC75E21 cDNA sequences exhibit 79% identity to one another, whereas the amino acid sequences are 85% similar and 81% identical to each other.

Example 8—Southern Blotting

To determine if either of the genes with phenylalanine decarboxylase activity could be responsible for the altered levels of phenylalanine-derived volatiles in 8-2-1 fruit with the *L. pennellii* introgression on chromosome 8, Southern blots on M82, 8-2-1 and *L. pennellii* genomic DNA were performed using cLEC73K23 as a probe. Polymorphisms between M82 and *L. pennellii* were observed with *EcoRI*, *EcoRV* and *ScaI* (Figure 10). With each of these enzymes the 8-2-1 restriction pattern was identical to *L. pennellii*, indicating that the cLEC73K23 gene in introgression line 8-2-1 came from the *L. pennellii* parent.

Example 9—Cloning of the *L. pennellii* cLEC73K23 Sequence

The nucleic acid coding sequence of the *L. pennellii* cLEC73K23 gene was obtained by PCR with primers from the 5' and 3' ends of the *L. esculentum* cLEC73K23. The correct 5' and 3' ends of the *L. pennellii* gene were then obtained by 5' and 3' RACE. The full-length coding

sequence of this gene and the amino acid sequence are shown in Figures 11A–11B (SEQ ID NO. 8 and SEQ ID NO. 9). The *L. pennellii* and *L. esculentum* cLEC73K23 cDNA sequences are 95% identical, whereas the amino acid sequences are 98% similar and 97% identical. An amino acid alignment of the *L. pennellii* and *L. esculentum* cLEC73K23 sequences and the *L. esculentum* cLEC75E21 sequences are shown in Figure 12.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Harry J.	Klee	Gainesville, Florida
Denise	Tieman	Gainesville, Florida

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Materials and Methods for Synthesis of a Flavor and Aroma Volatile in Plants

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☒ Customer Number 23557

OR

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 44

☒ Drawing(s) *Number of Sheets* 23

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number _____

☒ Other (specify) Sequence Listing (12 pages)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

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☐ No.

☒ Yes, the name of the U.S. Government agency and the Government contract number are: National Science Foundation Grant Number 7223426-12

Respectfully submitted,

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REGISTRATION NO. 38,261 *(if appropriate)*

Docket Number: UF-386CP

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Docket No. UF-386CP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Harry J. Klee, Denise Tieman
Docket No.	:	UF-386CP
For	:	Materials and Methods for Synthesis of a Flavor and Aroma Volatile in Plants

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